FORMATION OF METHYLMERCURY IN THE ENVIRONMENT

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Advanced Studies

The author attended the normal programme of events organised by the School of Chemistry, Leicester Polytechnic, for postgraduate students. These included fortnightly research meetings at which projects undertaken in the School of Chemistry were discussed; monthly research colloquia at which guest speakers from various institutions lectured on recent advances in chemical research; and workshops on glassblowing and basic computer programming.

DECLARATION

Unless otherwise stated, the work described in this thesis is the original work of the author. The author has not been registered for any other award of the CNAA, nor a univeristy, whilst registered for the CNAA degree of Doctor of Philosophy.

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ABSTRACT

AN INVESTIGATION OF FACTORS CONTROLLING LEVELS AND PATHWAYS OF METHYL-MERCURY IN THE NATURAL ENVIRONMENT

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The concept of environmental methylation of metal and metalloid elements is reviewed in an historical context, and the literature on the environmental methylation of one metal, mercury, is reviewed comprehensively. The recent use and emission of mercury in the United Kingdom are also reviewed and comparisons made with use in the United States, the largest consumer of mercury.

Analytical techniques for the determination of sulphide in environmental samples were examined, and one technique was chosen, modified and developed to suit the particular needs of this project. Existing methods for the determination of Eh value, methylmercury, total mercury and organic carbon contents of sediments also were developed. In addition, sampling and storage procedures for sediments were examined, and a method capable of preserving ambient methylmercury and sulphide levels in sediments was developed.

Surveys of methylmercury levels in sediments of three polluted estuaries - Carron, Clyde and Mersey - and some relatively unpolluted estuaries - in S.W. England - were undertaken. Sediment methylmercury concentrations occasionally were found to correlate with Eh values, organic carbon contents and total mercury levels in sediments; however, the strongest relationships were found between sediment methylmercury and sulphide concentrations.

Laboratory studies were undertaken to assess the effect of changes in the chemical environment of mercury on rates of methylmercury production in natural sediments. The relative importance of chemical and microbiological methylation processes in determining environmental methylmercury levels was also assessed. Finally, investigating a previously suggested route for loss of methylmercury from the sediment environment, the reaction between methylmercury and sulphide in a sediment matrix was examined: this was shown to lead to the evolution of dimethylmercury into the headspace above the sediment.

In consideration of the project as a whole, a close relationship between methylmercury and sulphide levels in sediments is demonstrated, and several factors are proposed to account for this observation.

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INTRODUCTION

Chapter 1

Environmental Methylation: Historical Aspects

Methylmercury is present in the environment owing to (1) direct discharge into the environment of methylmercury compounds used in industry and agriculture and (2) methylation of inorganic mercury present in the environment. The relative importance of these two routes in producing environmental levels of methylmercury varies with location and time. It may be noted, for instance, that the high concentrations of methylmercury in sediments of Minamata Bay (ppm range), Japan, in the 1960s -which resulted in the widely publicised outbreak of poisoning - was caused largely by the direct discharge of methylmercury in industrial effluent into the bay. (The Minamata incident gave rise to widespread concern about mercury in the environment, and is reviewed in more detail in Chapter 3.)

Another outbreak of poisoning caused by man-made methylmercury occurred in Iraq during 1972. In this instance, grain intended for sowing, and which had been dressed with methylmercury fungicide, was ingested by part of the population. This resulted in the worst recorded epidemic of organomercury poisoning and the victims numbered several thousand.

In the U.K., methylmercury compounds are prohibited from use as industrial slimicides and fungicides in agriculture and horticulture, and it can be assumed that methylmercury found in river and water systems of this country is present as a result of the environmental methylation of inorganic mercury. The discovery that inorganic mercury can be methylated in the aquatic environment was made in the 1960s, although the concept of environmental methylation of metal and metalloid elements was developed at a much earlier date. In this chapter a brief review of the historical

aspects of environmental methylation will be presented; this will be followed in Chapter 2 by a review of the recent uses of mercury and its compounds in the U.K.; Chapter 3 will review more fully the literature on the environmental methylation of mercury.

It is convenient to begin by defining the term "environmental methylation", as this is often confused in the literature with the term "biomethylation", resulting in semantic difficulties. There are six processes by which a methyl group may be transfered to a metal on exposure to an environmental situation:-

(1) Enzymatically, by the transfer of a methyl group within a cell of a living organism.

(2) Non-enzymatically, by reaction of a metal with a natural product, eg. a metabolite of an organism, having methylating properties.

(3) Reaction of a metal with a man-made methylating agent, e.g. Me_3Pb^+.

(4) Disproportionation of a partially methylated species, e.g.

$$3(Me_3Sn)_2S \longrightarrow 3Me_4Sn + \text{cyclo-}(Me_2SnS)_3$$

(5) Reduction of a one carbon fragment attached to a metal resulting in the formation of a methyl group, e.g.

$$-CH_2-OH \longrightarrow -CH_3$$

(6) Intra-molecular rearrangement involving transfer of a methyl group within a molecule, e.g. the formation of methylmercury from mercuric acetate exposed to daylight.

The term biomethylation may be applied legitimately to the first of the processes described. However, many workers also apply the term to the second of the processes

mentioned, although this process is, strictly speaking, non-biological. In this work the term environmental methylation will be used as an overall term to cover all possible mechanisms of environmental methylation, and the term biomethylation will be used only where there is direct evidence of an enzymatic process.

The concept of environmental methylation was developed in the nineteenth century. As early as 1824, Gmelin[1] mentioned the exhalation of a strong garlic odour on administration of potassium tellurite to animals, and in 1853 Hansen[2] described the same effect in man and stated that the odour resembled diethyl telluride, Et_2Te. However, it was not until 1894 that the intervention of methyl groups in these phenomena was discussed when Hoffmeister[1] stated, though without complete proof, that the "tellurium gas" was dimethyl telluride, Me_2Te. Hoffmeister considered that methyl goups were transferred to tellurium from tissues which possess the capacity for methylation. He stated, "in the presence of tellurium this is methylated, whereas under normal conditions methyl derivatives such as choline and creatine are produced". No particular compound was suggested as the source of the methyl group until 1913 when Riesser[3] considered that the methyl groups transferred to tellurium probably arose from choline ($(CH_3)_2N(OH)CH_2CH_2OH$) or betaine ($(CH_3)_3\overset{+}{N}CH_2COOH$). This suggestion was based partly on his observation that, when tellurite was heated with sodium formate and either choline or betaine hydrochloride, an odour resembling dimethyl telluride was evolved. Challenger[4] extended this work and demonstrated the formation of dimethyl selenide and dimethyl sulphide on heating sodium selenite and sulphite with pure betaine. These reactions were imitations at high temperature of environmental methylations.

It was Challenger who demonstrated the first environmental methylation of a metalloid element, namely, arsenic. The background to this work originated in the nineteenth

century and is recounted below.

In 1815, numerous cases of arsenical poisoning occurred in Germany owing to the use of domestic wall-papers, the pigments on which contained copper hydrogen arsenite. In 1839, Gmelin[1] noticed a garlic odour in "arsenical rooms" which he ascribed to a volatile arsenic compound liberated from the damp and mouldy wall-paper. In 1874, Selmi[1] suggested that the moulds produced hydrogen from the paper and paste which then gave rise to arsine, AsH_3. In 1891, Gosio[1] investigated this phenomenon further and produced a garlic odour by incubating arsenious oxide with moulds grown on potato-mash; this gas was incorrectly identified by Biginelli[1] as diethyl arsine. Finally in 1931, Challenger[5] positively identified "Gosio-gas", which he produced by incubating arsenic (III) compounds with cultures of the mould S. brevicaulis, as trimethyl arsine. The gas was identified following the characterisation of the compounds precipitated on passing the gas through Biginelli's solution (mercuric chloride in dilute hydrochloric acid); these were identified as $Me_3As.2HgCl_2$ and $Me_3As.HgCl_2$, the gas was thus trimethyl arsine (Me_3As). Some years later Challenger[1] stated that the methylation appeared to take place by transfer of a methyl carbonium ion from S-adenosylmethionine (SAM) in the mould, to a lone electron pair on arsenic (III). The reaction may be represented as the addition of a methylcarbonium ion (CH_3^+) to a neutral molecule, followed by expulsion of a proton:

$$\underset{(SAM)}{RR'\overset{+}{S}CH_3} + :As(OH)_3 \longrightarrow [RR'S \overset{+}{C}H_3:As(OH)_3]$$

$$\longrightarrow RSR' + [CH_3\overset{+}{A}s(OH)_3] \longrightarrow CH_3AsO(OH)_2 + H^+$$

Successive methylations produce the final product, trimethylarsine, viz:

$$As(OH)_3 \longrightarrow CH_3AsO(OH)_2 \longrightarrow (CH_3)_2AsO(OH)$$

$$\longrightarrow (CH_3)_3AsO \longrightarrow (CH_3)_3As$$

In his initial experiments, Challenger[6] also attempted a methylation of mercury by incubating mercuric oxide with S. brevicaulis. His failure to produce methylmercury is explicable as mercury (II) does not possess a lone pair of electrons and hence does not behave as a nucleophile towards carbonium ions.

The environmental methylation of mercury finally was demonstrated in the 1960s. Three observations led to this discovery:

First, it was found that most of the mercury present in fish was present in the methyl form[7]; second, it was discovered that inorganic mercury when added to aquarium sediments was partly converted to methylmercury[8]; and third, it was found that methylcobalamin ($Me(B_{12})$) - utilising methanogenic bacteria could methylate mercury in sediment environments[9]. The methylation of Hg (II) by $Me(B_{12})$ proceeds by the transfer of a methyl carbanion,

$$Me(B_{12}) + Hg^{2+} \xrightarrow{H_2O} MeHg^{+} + H_2O(B_{12})^{+}$$

Of the three main natural methylating systems, $Me(B_{12})$, SAM and ^{5}N-methyltetrahydrofolate, only $Me(B_{12})$ can transfer a methyl group as a carbanion, and hence in principal is the only species capable of methylating Hg (II).

Since the 1960s, the formation of methylmercury from inorganic mercury present in sediments has been demonstrated many times; this work will be reviewed in Chapter 3.

In the last ten years much interest has focused on the environmental methylation of tin and lead. This interest derived both from (1) concern about the toxicity of methylmetal species, and the possibility that they may become widely distributed in the environment, and (2) the development of sensitive analytical techniques capable of detecting low concentrations of these species (methylated tin and lead compounds have been found in the environment

in the pg g^{-1} and ug g^{-1} range respectively)[10, 11]. In general, methylated organometallic species are more toxic than the inorganic compounds from which they derive. This arises in part from their greater solubility (compared to metallic ions) in lipid tissue, leading to much longer residence times in organisms. Although the environmental concentrations of these compounds are low, they may still be significant; continuous formation of these compounds in the environment may result in food chain effects leading to much higher concentrations in organisms.

There are no reports of the formation of tetramethyltin (Me_4Sn) from the incubation of Sn (O), Sn (II) and inorganic Sn(IV) compounds in natural sediments, although the formation of methyltin cations has been reported.[12] However, the production of Me_4Sn following the addition of trimethyltin salts to natural sediments has been demonstrated.[12] The exact mechanism leading to the formation of Me_4Sn is not known, but two probable mechanisms are (1) simple disproportionation of the trimethyltin salts[13,14] and (2) reaction of the trimethyltin salts with sulphide, naturally occurring in sediments, leading to the formation of bis-trimethyltin sulphide ($(Me_3Sn)_2S$) which is then followed by the dismutation of this compound[15]:

$$3(Me_3Sn)_2S \longrightarrow 3Me_4Sn + \text{cyclo-}(Me_2SnS)_3$$

The reaction proceeds by methyl migration between tin atoms followed by cyclization of the dimethyltin intermediate. An analogous reaction also occurs with trimethyllead and monomethylmercury compounds; this is discussed further in Chapter 3. Laboratory experiments have shown that Sn (O) and Sn (II) salts will react with the natural methylating agent, iodomethane, to produce methylated tin products and a methylation of tin (II) by $Me(B_{12})$, also a natural methylating agent, has been demonstrated[12,16]. Finally, the formation of Me_4Sn following the incubation of hydrated tin (IV) chloride with a tin-resistant

Pseudomonas strain has been reported[17,18].

The subject of the environmental methylation of lead is controversal. Although there is general agreement that trimethyllead salts are converted to tetramethyllead (Me_4Pb) in the environment, there is no consensus about what proportion of the product arises from mere disproportionation (both simple disproportionation and dismutation through the sulphide route) and what proportion, if any, arises from genuine biological methylation. The methylation of lead (II) in the environment also remains an open question. There have been a number of reports from groups who have been unable to detect lead (II) methylation in various microorganisms or sediment media[19,20,]. The environmental methylation of lead (II) may be precluded by the high instability of monomethyllead ($MePb^{3+}$) species, although, theoretically, there is no reason why lead (II) may not be methylated by a natural agent, if the rate of methylation of $MePb^{3+}$ to the more stable dimethyllead (Me_2Pb^{2+}) and trimethyllead (Me_3Pb^{+}) proceeds at a faster rate than the rate of decomposition. The natural metabolite, iodomethane, has been shown to react with lead (II) salts to form Me_2Pb^{2+} and Me_3Pb^{+} species, although the fully methylated product, Me_4Pb, was not detected. The formation of Me_4Pb from the reaction of Pb(0) with iodomethane has been demonstrated, however[21,22].

The possibility of other metals undergoing methylation in the environment has been investigated. Arsenic methylation from freshwater lake sediments has been demonstrated recently with arsine or arsonic acid derivatives being identified[23,24,25]. Small amounts of dimethylthallium (III) have been produced from the incubation of thallium (I) in anaerobic sediments, although there is at present no other environmental evidence for thallium methylation[14]. There is also some evidence to suggest that methylation of platinum in the environment could occur, a water-stable

methyl platinum species having been isolated from the reaction of $Me(B_{12})$ with $PtCl_6^{2-}$ and $PtCl_4^{2-}$ in aqueous solution(26,27,28). Finally, it is possible that cadmium may be methylated by a particular strain of Pseudomonas; however, methyl cadmium compounds are very unstable in water and are thus unlikely to be detected in the environment(29,30,31).

In conclusion, it can be said that the concept of environmental methylation was developed by Challenger in the 1930s following his investigation of phenomena which had been observed in the nineteenth century. In recent years an impetus to further research in the subject was provided by outbreaks of methylmercury poisoning in Minamata and elsewhere, and by the development of sensitive analytical techniques capable of detecting the very low concentrations of methylmetal species present in the natural environment.

Chapter 2

Sources and Concentrations of Mercury

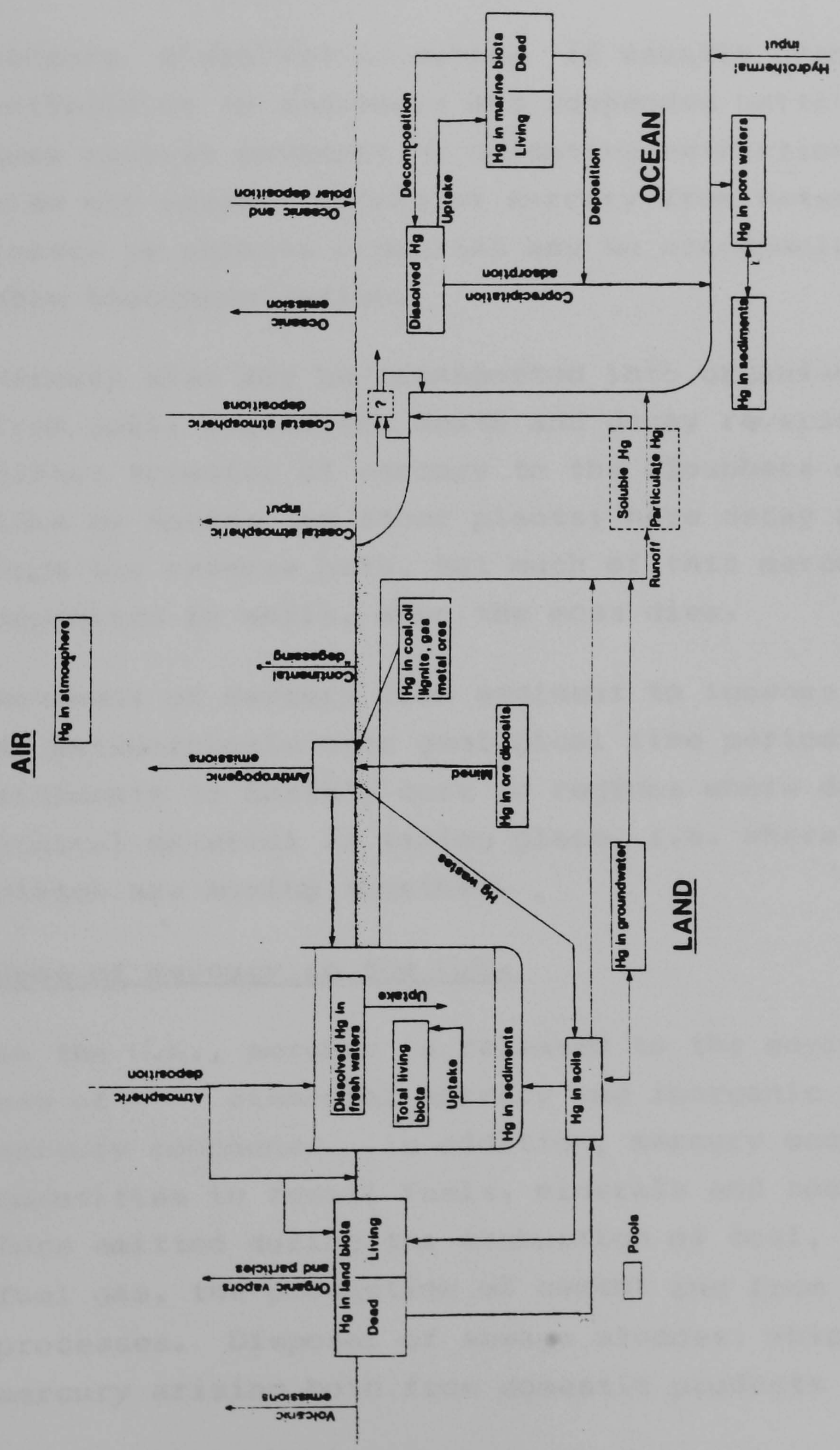
Mercury is present in the sediment environment as a consequence of both natural emissions of mercury and inputs arising from man's activities. This chapter outlines natural sources of mercury and then reviews recent use of the element, with particular reference to use in the United Kingdom (U.K.). Finally, concentrations of mercury in sediments of some rivers and estuaries of the U.K. are considered in the context of this discussion. Comparisons are also drawn with mercury levels in sediments of some selected water systems of Europe, North America and Asia.

The Global Cycle of Mercury

A discussion on the natural inputs of mercury to the sediment environment must include a consideration of the global cycle of mercury, as there is a continuous exchange of mercury between the atmosphere, hydrosphere, lithosphere and biosphere (Fig. 1)

The major natural sources of mercury are volcanic action and degassing of Earth's crustal materials[32,33] (the overall mean crustal concentration of mercury is estimated at 0.07 ppm). These processes release mercury to the atmosphere in elemental and particulate form, i.e. as vapour and as finely-divided solid mercury compounds. Up to 150,000 tonnes of mercury may be mobilised each year in this way[34]. Additionally, natural erosion of rocks and mineral deposits by weathering transport mercury into the hydrosphere (rivers, lakes and oceans) at a rate variously estimated from 230 - 5000 tonnes annually[35,36]. Submarine leaching, erosion and volcanic activity must also transport mercury from sub-marine rocks and sediments and from Earth's core to the seas, but the total quantity involved

Fig. 1.


The Global Mercury Cycle
AIR
LAND
OCEAN
Hg in atmosphere
Anthropogenic emissions
Natural "degassing"
Continental
Hg in coal, oil lignite, gas metal ores
Mined
Hg in ore deposits
Hg wastes
Hg in groundwater
Hg in soils
Hg in sediments
Dissolved Hg in fresh waters
Total living biota
Uptake
Atmospheric deposition
Organic vapors and particles
Volcanic emissions
Hg in land biota
Living
Dead
Runoff
Soluble Hg
Particulate Hg
Coastal atmospheric input
Coastal atmospheric depositions
Oceanic emission
Oceanic and polar deposition
Hg in marine biota
Living
Dead
Decomposition
Uptake
Deposition
Dissolved Hg
Coprecipitation adsorption
Hydrothermal input
Hg in pore waters
Hg in sediments
Pools

does not appear to have been estimated.

Movement of mercury from the atmosphere to marine and freshwaters occurs in rain and dustfall and these together may amount to approximately 50,000 tonnes of mercury per year[37]. A reverse movement occurs by evaporation, but to a much smaller extent, possibly about 2,000 tonnes per year[37].

Mercury dissolved in waters is usually adsorbed preferentially on to sediments and suspended particulate materials; some reverse movement by oxidative extraction or absorption also may occur. Uptake of mercury from waters and sediments by aquatic organisms may be accompanied by considerable bioconcentration.

Mercury also may be transported into organisms via uptake from soil; excretion, death and decay reverse the process. Direct transfer of mercury to the biosphere occurs in uptake by mosses and other plants; here decay and evaporation form the reverse path, but much of this mercury will be deposited in soils, when the moss dies.

Movement of mercury from sediment to igneous rock occurs by metamorphosis over geological time periods, and from sediments to Earth's core in regions where destruction of crustal material is taking place, i.e. where tectonic plates are moving together.

<u>Uses of Mercury in the U.K.</u>

In the U.K., mercury is released to the environment through use of elemental mercury and inorganic and organic mercury compounds. In addition, mercury occurs in trace quantities in fossil fuels, minerals and rocks and is therefore emitted during the combustion of coal, oil or fossil-fuel gas, the production of cement and from metallurgical processes. Disposal of sewage sludges, which may contain mercury arising both from domestic products and industrial

losses, also releases mercury to the environment.

The most recent data available detailing the use of mercury in the U.K. was published in 1976[38]; since then there has been no systematic collection of data on the subject. Most of the details on the use of mercury given in this thesis are taken from a 1976 HMSO publication[38] and may be somewhat historic in terms of present day use. It is likely that the quantities of mercury used in the U.K. will have declined during the last eight years owing to the closure of chloralkali plants and the effects of the economic recession.

The major use of mercury in the U.K., and indeed in the world, occurs in the simultaneous production of chlorine and caustic soda by the electrolysis of brine (the chloralkali process) utilizing mercury cells. The process employs a cell which consists of a metallic mercury cathode and a graphite anode. The chloralkali industry was responsible in 1975 for emissions of about 18 tonnes of mercury to water, about 22 tonnes to the atmosphere, 1.4 tonnes in the caustic soda produced and 73 tonnes in waste sludges.

The use of mercury compounds in paints has been reviewed[39]. The largest proportion of the total use occurs in general decorative paints (mostly emulsion paints), in which mercury compounds (usually phenyl mercury compounds) are incorporated as general biocides at concentrations of about 0.001-0.05 per cent, expressed as mercury by weight. Much of this mercury, which amounts to 30 tonnes annually, must eventually be released to the environment. Mercury compounds (about 4 tonnes annually) are used as antifouling formulations in marine paints, but here the mercury concentration is much higher, about 2-5 per cent by weight. The use of mercury compounds in adhesives, e.g. wallpaper pastes, has declined in recent years and is now very small.

Mercury catalysts are used in the manufacture of elastomeric

polyurethanes and here traces of the catalyst end up in the product. The estimated annual consumption of mercury in the U.K. in this application is 2 tonnes; ultimately most of this will be disposed of by landfill. The dyestuffs industry also uses mercuric sulphate catalysts in the manufacture of anthraquinone sulphonic acid dyestuffs, some 5-10 tonnes of mercury equivalent as oxide being used annually in the U.K. Some of the mercury goes into the product and the rest into waste sludges. Mercury catalysts are no longer used in the U.K. in the production of vinyl chloride and vinyl acetate.

The production of primary batteries, now widely used in domestic, industrial, office and medical equipment, accounted for a total of 80 tonnes of mercury in 1975; other electrical apparatus and control instruments accounted for a further 14 tonnes.

Mercury amalgams have been used as dental fillings for about 150 years and today approximately 30 tonnes of mercury are used annually for this purpose. Although mercury vapour and particulates are released to air during the preparation of fillings and the drilling out of old fillings, it has been shown that this does not result in a health hazard for patients or dental staff[38].

The use of mercury compounds in agriculture and horticulture has been restricted considerably in recent years. Under the Pesticides Safety Precautions Scheme, recommendations for the following product uses were withdrawn as from 1st January 1981 for products leaving manufacturer's premises:-

Mercuric chloride in agriculture, horticulture and forestry.
Phenyl mercury acetate in agriculture and horticulture.
Phenyl mercury salicylate aerosol in agriculture and horticulture.
Methylmercury liquid seed dressing in agriculture and horticulture.

Restrictions have also been placed on the use of the following compounds:-

Mercuric oxide fungicidal paints in agriculture.
Mercurous chloride in agriculture, horticulture and home garden.
Aryl mercury foliage sprays in agriculture and horticulture.
Aryl mercury dry seed treatments in agriculture and horticulture.
Aryl mercury liquid seed treatments in agriculture and horticulture.
Organomercury dips for sheep.

However, between 10 and 20 tonnes of mercury compounds are still used annually for agricultural purposes in the U.K.[40].

Combustion of coal may release between 12 and 36 tonnes of mercury to air annually in the U.K. This probably becomes widely dispersed and diluted. Oil combustion probably releases about 5 tonnes of mercury annually and cement production about 2 tonnes; this mercury is also likely to be widely dispersed, eventually being deposited on the ground or in water. Combustion of fossil-fuel gas also probably releases a few tonnes of mercury to the atmosphere each year.

Sewage sludges may contain mercury concentrated from industrial , domestic and agricultural effluent. Sludges are disposed of by dumping at sea, by landfill or incineration or are used as fertilisers. About 10 tonnes of mercury enter the sea annually around England and Wales as a consequence of sludge dumping from ships. The total quantity of mercury in sludges disposed of each year by landfill, incineration and use as fertiliser is also probably about 10 tonnes.

Non-ferrous metal ores almost invariably contain traces of

mercury. Processing releases part of the mercury content to the atmosphere and this may total up to about 5 tonnes annually in the U.K. Iron and steel production similarly release only a few tonnes of mercury annually.

Mercury is also lost to the environment arising out of its use in medicinal and pharmaceutical products and from miscellaneous laboratory uses such as occur in barometers, thermometers and vacuum pumps; however, these losses are not reliably calculable.

The use of mercury in the U.K. can be contrasted with use in the U.S.A. (Table 1), which, in 1975, was the largest consumer of mercury[41].

Table 1

Use of Mercury in the U.S.A. and U.K. in 1975 (tonnes)[a]

USES	U.S.A.	U.K.
CHLORALKALI PLANTS	525 (29.9)	283 (57.1)
ELECTRICAL GOODS AND CONTROL APPARATUS	744 (42.4)	94 (19.0)
PAINTS	239 (13.6)	34 (6.9)
DENTAL PREPARATIONS	81 (4.6)	30 (6.0)
AGRICULTURE	21 (1.2)	28 (5.6)
GENERAL LABORATORY USE	12 (0.7)	10 (2.0)
CATALYSTS	29 (1.6)	9 (1.8)
PHARMACEUTICALS	15 (0.9)	2 (0.4)
OTHER	88 (5.0)	6 (1.2)
TOTAL	1754	496

[a] Numbers in parentheses are percentages of total use.

Table 1 shows that the two principal uses of mercury in the U.S.A. and U.K. (which accounted for 72.3 and 76.1 per cent of total use respectively, in 1975) have been in the manufacture of electrical goods and in the production of caustic soda and chlorine. For the other major uses listed, Table 1 shows that proportionate use of mercury in both countries has been similar, although mercury appears to have been used more extensively in agriculture in the U.K. Comparable end use data are not available for other countries[41], but Table 1 should give a general picture of the applications of mercury in industrialised nations.

Between 1965 and 1975, the U.K. used between 235 and 788 tonnes of mercury each year; this compares with a world-wide use of about 9,000 tonnes annually during the same period. In 1975 it was estimated that, world-wide, some 7,500 tonnes of mercury were released to the environment from industrial and agricultural sources[42], a small figure compared to the amount of mercury released by natural processes (approximately 150,000 tonnes). However, more recently, it has been estimated that between 25 and 30 per cent of the atmospheric mercury burden is due to anthropogenic emission, and that generally the mercury burden of rivers (water plus bottom and suspended sediments) has increased by a factor of four when compared with pre-man levels[43]. The main significance of anthropogenic emissions, however, is that they are localised and may give rise to high concentrations of mercury in some local part of the environment.

It is doubtful if future use and emission of mercury can be estimated reliably, although it seems clear that use in the chloralkali industry and agriculture will continue to decline, both in the U.K. and abroad, as diaphragm cells replace mercury cells for the manufacture of chlorine and caustic soda, and less toxic non-mercury containing fungicidal compounds are developed. However, one forecast

suggests that total mercury emission in industrialised countries may remain static at 1975 levels, and that industrialisation of underdeveloped countries could increase world-wide emission from 7,500 tonnes in 1975 to 10,000 tonnes in the year 2000 (44).

Mercury Levels in Sediments of Some U.K. Rivers

To conclude this chapter, levels of mercury in some U.K. river sediments are compared with reference to the pollution inputs received by the water systems.

There have been many studies on mercury levels in U.K. river sediments, and the results of some of the most recent surveys are reported in Table 2.

Table 2

Mercury Levels in Sediments of Some U.K. Rivers

Location	Concentration (ug g^{-1})	Reference
River Dart	0.01 - 0.55	(45)
River Wyre	0.11 -10.20	(46)
River Mersey	< 0.05 - 4.83	(47)
River Clyde	< 0.05 - 3.68	(47)
River Carron	0.05 - 3.95	(48)
River Plym	0.02 - 0.49	(49)
River Tamar	0.20 - 1.50	(50)

The data reported in Table 2 represents the total concentration of all forms of mercury present in the sediment matrix.

The Mersey, Wyre and Carron have received specific inputs of mercury from industrial operations. The Mersey and Wyre have received chloralkali effluent and the Carron has received mercury-containing effluent from a dyeworks. The other rivers listed in Table 2 have received only small

amounts of mercury from inputs of domestic sewage and general industrial effluent, the Clyde having received the greatest quantity of general pollutants. The Dart, Plym and Tamar drain a mineralised catchment area, where until the turn of the century metals were mined; however, there are no significant quantities of mercury ore in the catchment area.

Not surprisingly, the highest levels of mercury are found in the sediments of rivers which receive specific inputs of mercury, average concentrations of 3.00, 2.59 and 2.23 ug g^{-1} of mercury have been recorded for Wyre[46], Carron[48] and Mersey[51] sediments respectively. However, high sediment mercury levels may also be found near sewage outfalls in rivers which receive no specific mercury input, for instance, the Clyde, where sediments adjacent to a sewage outfall have been found to contain as much as 3.68 ug g^{-1} mercury, although the average concentration of mercury in Clyde sediments is about 0.60 ug g^{-1}[47]. Finally, sediments in the River Dart, which is relatively unpolluted, have been found to contain an average of 0.22 ug g^{-1} mercury[45]. Thus, it may be said that sediments of U.K. rivers which have suffered badly from mercury pollution contain, on average, mercury levels which are approximately 10 x greater than mercury concentrations found in sediments of relatively unpolluted rivers.

It may be noted that mercury levels in sediments of polluted U.K. rivers are low compared with mercury levels which have been found in sediments of some polluted water systems in Europe, North America and Asia (Table 3).

The high levels in the Monte Amiata region have been caused by mining of mercury ore. The Wabigoon River System has received specific mercury inputs from a chloralkali plant and a paper mill. Minamata Bay has received mercury-containing effluent from an acetaldehyde manufacturing plant. The Seine and Rhine have received heavy loads of

Table 3

Mercury Levels in Sediments of Some Selected European, North American and Asian Water Systems.

Location	Concentration (ug g^{-1})	Reference
Seine, Mantes, France	9.8 - 15.8	(52)
Monte Amiata, Italy	64 - 288	(52)
Upper Rhine, France	0.12 - 58	(53)
Lower Rhine	5 - 17	(54)
Yessel, Holland	12	(54)
Wabigoon, Canada	0.56 - 66	(55)
Minamata, Japan	12 - 2010	(56)

general pollutants.

Methylmercury levels in sediments of some of these water systems will be considered in Chapter 4.

Chapter 3

Environmental Methylation of Mercury

Mercury in all its forms is toxic to human and animal life, and methylmercury is particularly hazardous due to a combination of its lipid solubility and ionic properties leading to an high ability to penetrate membranes in living organisms.

Two discoveries made in the 1960s led to concern about methylmercury in the environment. First, it was found that methylmercury poisoning had been the cause of 'Minamata disease', outbreaks of which had occurred in Japan at Minamata (1953-1960) and Niigata (1965). These incidences of poisoning were caused by the ingestion of fish which had accumulated high concentrations of methylmercury through the food chain. Methylmercury was present in the aquatic environment at these locations as a result of the discharge of the compound in effluent from acetaldehyde and vinyl chloride manufacturing plants. The solution to the Minamata problem was, therefore, the cessation of the input of methylmercury into the water systems. However, a potentially more serious problem was recognised following Westoo's[7] discovery that fish could accumulate high concentrations of methylmercury in areas where there are no specific inputs of methylmercury. This apparent paradox was resolved when Jensen and Jernelov[8] found that inorganic mercury present in natural sediments could be converted into methylmercury.

The methylation of inorganic mercury in the sediment environment has been demonstrated many times since the publication of Jensen and Jernelov's work, and, indeed, during the last 15 years considerable research has been aimed at understanding and describing mechanisms and rates

of methylmercury production in the aquatic and sediment environment. Some of the published research results are conflicting, although points of consensus have emerged. The aim of this chapter is to draw out the salient points from the plethora of literature on the subject of the environmental methylation of mercury.

Sediment Incubation Experiments

Jensen and Jernelov's[8] research involved the incubation of fresh-water aquaria and fresh-water lake sediments which had been inoculated with mercuric chloride. In one experiment, lake sediments, amended with mercuric chloride to concentrations of 10 and 100 ug g^{-1}, were incubated and analysed periodically for methylmercury content; the results are shown in Fig. 3. In another experiment, a series of lake sediments was amended with varying concentrations of mercuric chloride and analysed after 7 days incubation; the results are shown in Fig. 2. The results of both these experiments demonstrated low but definite yields of methylmercury (less than 1 per cent). The authors also incubated a sterilized sediment which had been inoculated with mercuric chloride, but failed to observe the production of methylmercury; they, therefore, concluded that the methylation process had a biological origin. Further evidence in support of this conclusion was provided by the failure of the sediment amended to 1000 ug g^{-1} with mercuric chloride to produce methylmercury (Fig. 2), a possible explanation being that the high concentration of mercury had exceeded the threshold value for the operation of the microbes. Jensen and Jernelov[8] also demonstrated the formation of dimethylmercury (Me_2Hg) from dead fish which had been incubated in anaerobic conditions.

Further incubation experiments have elucidated the conditions favourable to the methylation of mercury in the sediment environment. Bishop and Kirsch[57] found that methylmercury production in an anaerobic environment rose with

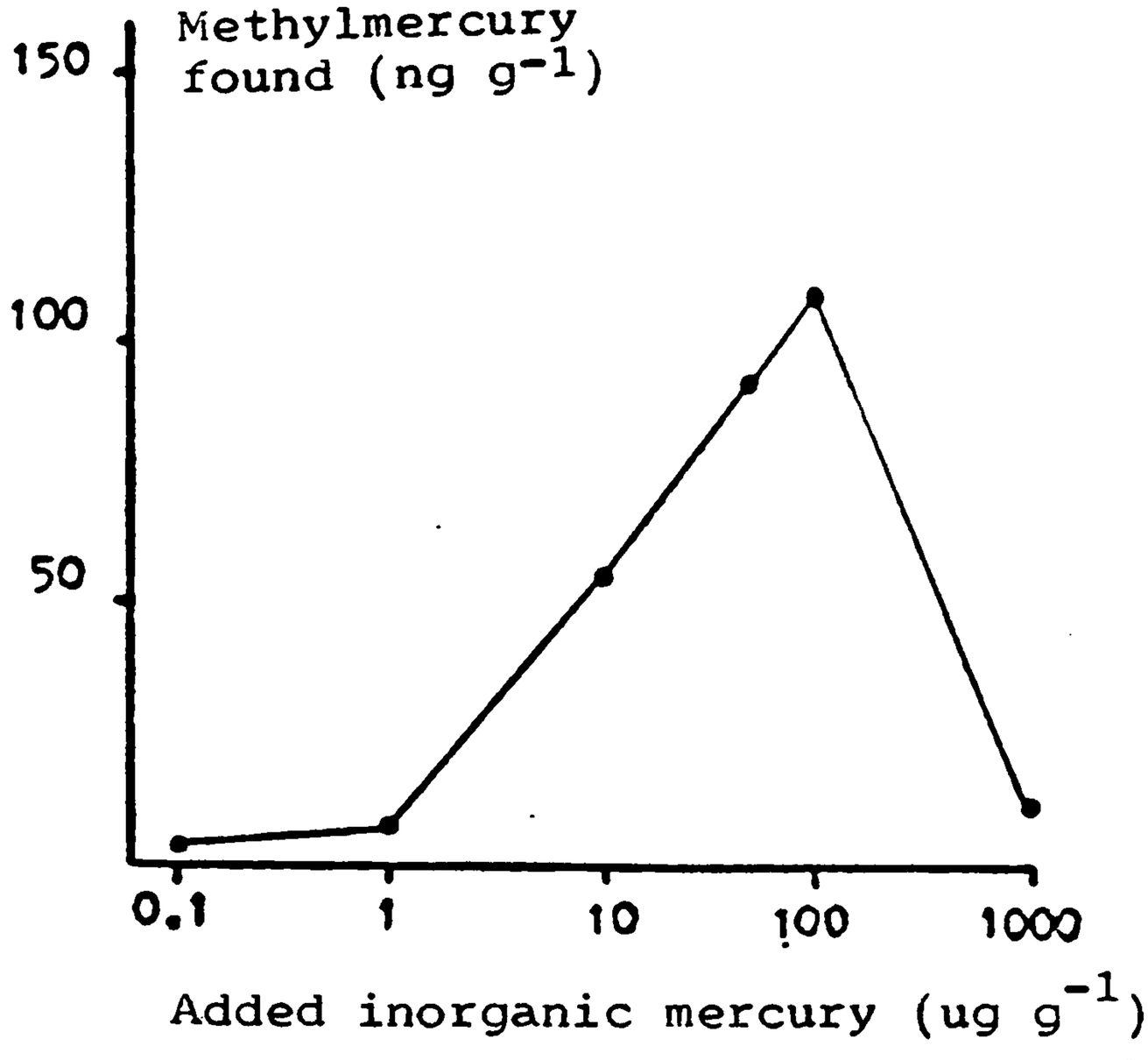


Fig. 2. Concentraion of methylmercury found in sediments after addition of inorganic mercury. Incubation for 7 days[8].

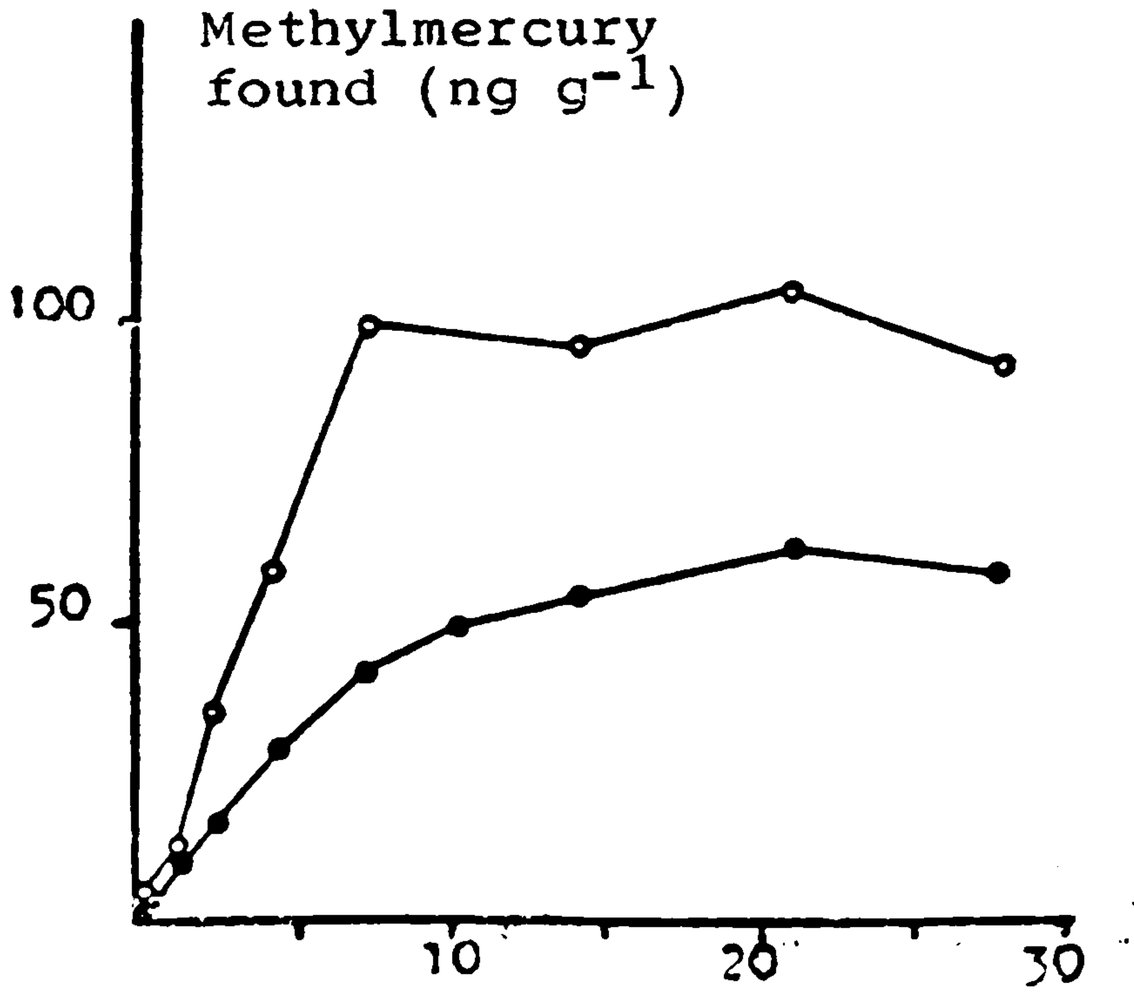


Fig. 3. Concentration of methylmercury found in sediments after addition of 10 (●—●) or 100 (o—o) ug g^{-1} of inorganic mercury[8].

increasing inorganic mercury dosage, increasing temperature, enrichment of methanogenic bacteria and supplementation with organic nutrients. They also concluded that little or no dimethylmercury was produced. Langley[58] observed that methylation rates in river sediment were dependent on total mercury concentration, microbial activity, organic matter concentration and pH in the sediment. Olsen and Cooper[59] studied methylation rates in aerobic and anaerobic sediments. They found that high organic carbon content sediments gave the highest methylation rates in both aerobic and anaerobic environments. They also showed that increased inorganic mercury input caused greater methylation rates. Finally, they concluded that methylation appears to proceed at much greater rates in sediments maintained under anaerobic conditions than those incubated with access to air. Baker et al.[23] found a highly pH dependent methylation of mercury in an oligotrophic lake sediment, methylation occuring only in the pH range of 5.5-6.5. Blum and Bartha[60] found that saline environments produce less methylmercury than equivalent non-saline regions. Finally, it has been suggested that methylation rates are dependent on the chemical form of mercury added to the sediment. Thus Jacobs and Keeney[61] found that sediments doped with phenylmercuric acetate produced greater yields of methylmercury than sediments doped with an equivalent amount of mercuric chloride, and similarly Fagerstrom and Jernelov[62] found that sediments doped with mercuric chloride produced greater yields of methylmercury than sediments doped with an equivalent amount of mercuric sulphide. Fagerstrom and Jernelov[62] reported that the yields of methylmercury from mercuric sulphide are very low and that the reaction proceeds under aerobic conditions, presumably after the sulphide first is oxidised to sulphate, making Hg^{2+} available.

From the preceding studies it is apparent that methylation of mercury can occur in natural sediments and that the

rate of methylmercury production is small and dependent on a number of factors, some of which are inter-related, these include: the chemical form of mercury, mercury concentration, temperature, organic content, pH, Eh and microbial activity in sediments.

Microbial Methylation and Demethylation

The involvement of microorganisms in the generation of methylmercury species in the environment has been investigated extensively. Here the work of Wood has been seminal, and his contribution and that of others is now reviewed below; the mechanistic aspects of this work are reviewed in the next section of this chapter.

The involvement of microbes in producing methylmercury species in the environment was first demonstrated by Wood[9] when he produced a methylation by cell-free extracts from methanogenic bacteria. Subsequently, Yamada and Tonomara[63] found that an anaerobe isolated from soil (clostridium cochlearium) could methylate up to 40 ppm of added inorganic mercury. Vonk and Kaars Sijpesteijn[64] demonstated that several pure cultures of bacteria could aerobically methylate mercuric chloride. They also found that aerogenes and E. Coli anaerobically methylated mercury but at a lower rate than in aerobic systems. Edwards and McBride[65] observed methylation in anaerobic mixed cultures from human faeces and Hamdy and Noyes[66] performed methylation experiments on a mercury resistant strain of E. aerogenes. However, Reisinger et al.[67] failed to produce methylmercury from the incubation of Hg (II) with a pure culture of E. Coli and mixed cultures of methanogenic bacteria.

The role of microorganisms in demethylating methylmercury species in the environment also has been well documented. Furukawa et al.[68] demonstrated that a bacterial strain of the genus Pseudomonas, isolated from soil, was able to

decompose methylmercuric chloride to methane and elemental mercury. Spangler et al.[69] found 30 bacterial cultures which could aerobically degrade methylmercury and 21 cultures which could anaerobically degrade methylmercury. Billen et al.[70] also found that methylmercury could be decomposed under both anaerobic and aerobic conditions in the presence of bacterial cultures obtained from river sediments. McCarty[71] concluded that methylmercury added to aerobic and anaerobic cultures degraded faster under aerobic conditions. Generally speaking, it appears degredation of methylmercury proceeds at a faster rate in aerobic environments. Hence, apparent differences in methylmercury production between aerobic and anaerobic sediments may be more apparent than real, due to higher demethylation rates in aerobic sediments[59].

Billen[70] has suggested that methylmercury production and degredation in sediments may be an equilibrium process, perhaps as a consequence of some detoxification mechanism producing a steady state concentration of methylmercury tolerable to the microbes. Recently, Ramamoorthy et al.[72] have demonstrated that the physiological state of microbes in aquatic systems can greatly affect the way in which they deal with mercury compounds. The authors, working with E. Coli, Pseudomonas fluorescens and the blue-green alga Anabaena flos-aquae, found that growing bacterial cells transformed methylmercuric chloride to elemental mercury, and, that living but non-growing bacterial and algal cells also demethylated methylmercury. However, dead bacterial cells were found to methylate mercuric chloride.

Theoretical Mechanisms for Methylation and Demethylation Processes with Biological Origins

Methylation of inorganic mercury has been shown for direct biological reactions (enzymatic methylation) and for indirect biological reactions (nonenzymatic methylation)[73].

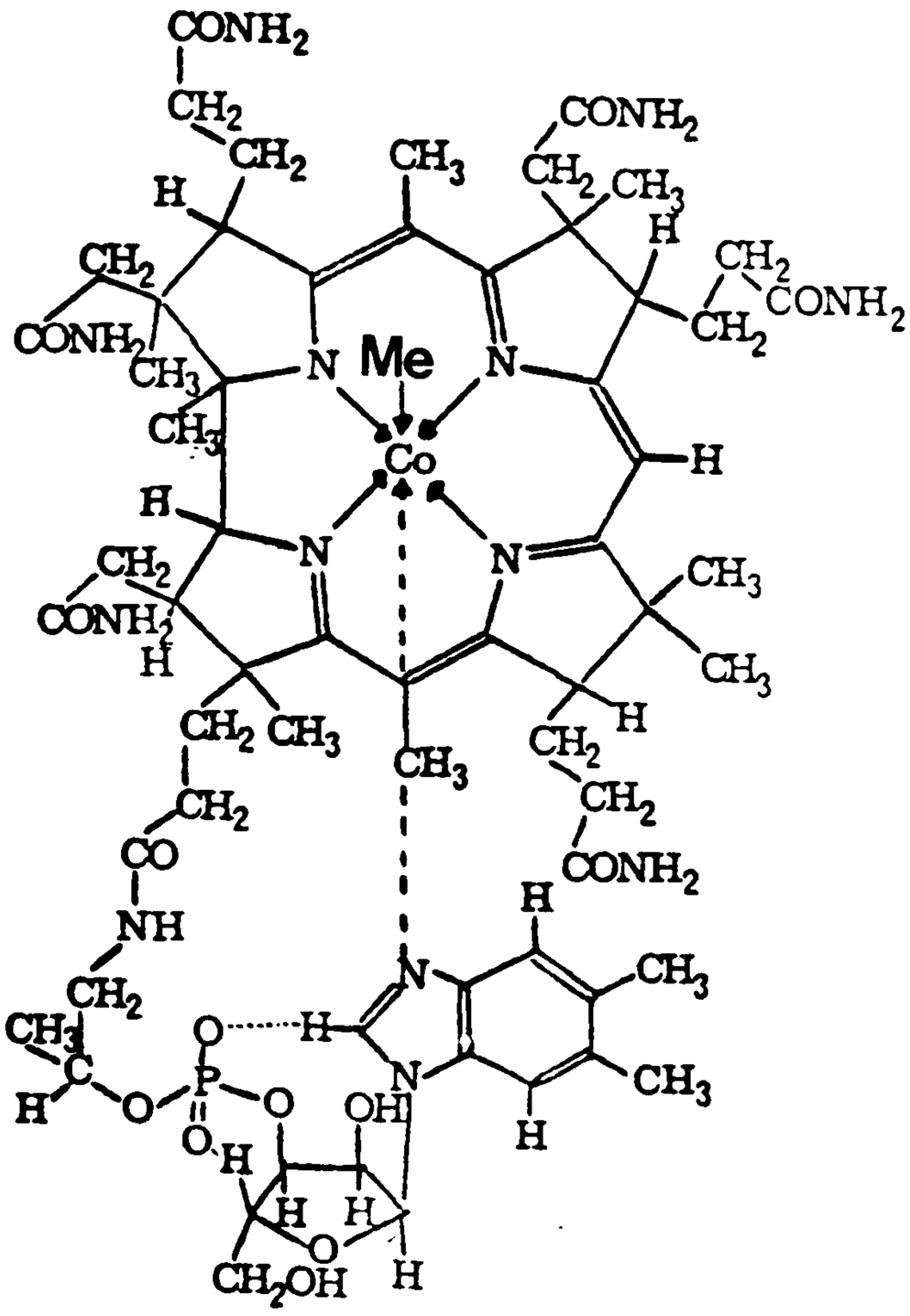
Enzymatic methylation requires the presence of actively metabolising organisms, whereas nonenzymatic methylation requires only the products (methyl donors) of active metabolism.

Nonenzymatic methylation of mercury with methylcobalamin ($Me(B_{12})$), a vitamin B_{12} analogue, has been studied by a number of workers. $Me(B_{12})$ may be simplistically described as an octahedrally co-ordinated cobalt (III) species with a cobalt methyl group bond (Fig. 4). Wood[9] was the first to propose that the methyl group bound to the cobalt atom can be donated to mercury. Imura et al.[74] and Bertilsson and Neujar[75] studied the reaction between $Me(B_{12})$ and mercuric ion and concluded that methyl transfer occurs with ease. A mechanistic study of the reaction was completed by Chu[76]; his results are summarised in Fig. 5. Essentually Chu proposed that, in the presence of free mercuric ion, an equilibrium is establised between the base on and base off form of $Me(B_{12})$, and that reaction of unionised mercury occurs with the base off species of $Me(B_{12})$ to form the products, methylmercury and aquo cobalamin ($H_2O(B_{12})$). However, if no free mercuric ion is present in the reaction mixture - due to the presence of complexing reagents, for instance - Chu concluded that the methylation reaction proceeds by a one step mechanism. A detailed kinetic study of the reaction between $Me(B_{12})$ and mercuric chloride was published by Craig and Morton[77]. The authors found that the mechanism of the reaction involved a carbanion transfer from base on $Me(B_{12})$ to mercuric chloride.

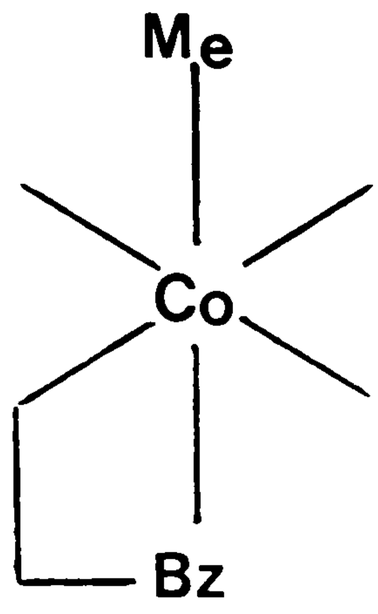
Wood[78] has proposed a number of mechanisms by which mercury can be enzymatically methylated. The first mechanism involves the cobalamin-dependent N^5-methyltetrahydrofolate-homocysteine transmethylase (methionine synthetase) enzyme. Some anaerobes and facultative aerobes use methionine synthetase to synthesise methionine from homocysteine. Microbes that utilize the

Fig. 4.

The Structure of Me(B_{12})



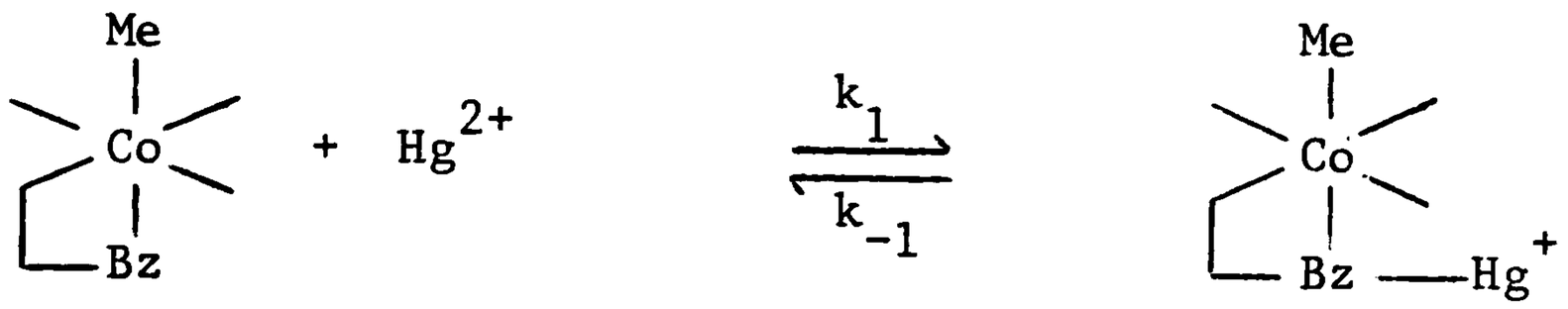
Simple representation of Me(B_{12})

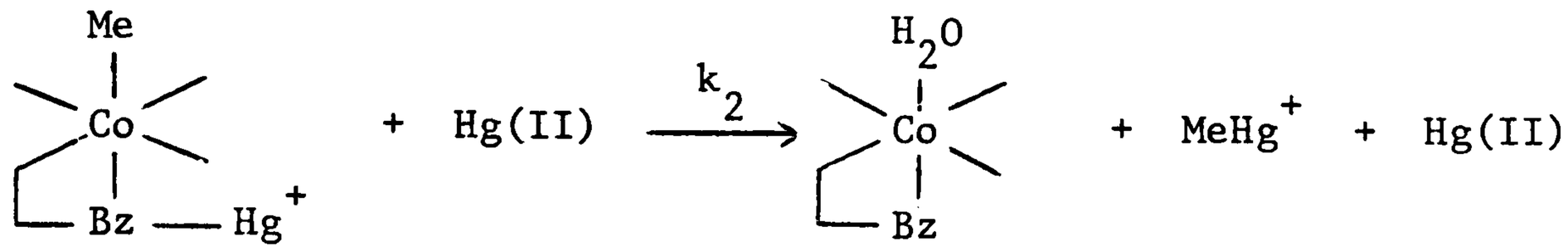


B_z = 5,6-dimethylbenzimidazole

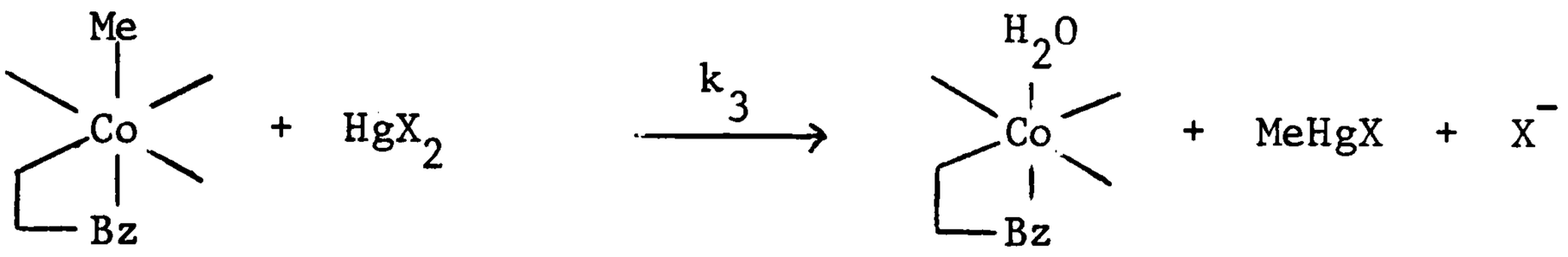
Fig. 5

<u>Two Step Reaction.</u> Occurs when $[Hg^{2+}]$ is high.





<u>One Step Reaction.</u> Occurs when $[Hg^{2+}]$ is negligible.



<u>The Reaction Mechanism Proposed by Chu</u>

enzyme system are capable of producing methylmercury; the reaction is summarised in Fig. 6.

The second enzymatic methylation mechanism that Wood discusses is the acetate synthetase system. Anaerobic organisms that synthesise acetic acid from carbon dioxide using this enzyme can produce methylmercury via the scheme presented in Fig. 7.

A third enzyme system, methane synthetase, which is common in anearobic ecosystems such as lakes and benthic regions, also has been implicated by Wood as a potential producer of methylmercury. However, Edwards and McBride[79] have provided strong evidence to the contrary. The authors found no correlation between methane biosynthesis and methylmercury synthesis, and, furthermore, they failed to isolate B_{12} type enzymes from methane bacteria.

Landner[80] has proposed a mercury methylation model for Neurospora crassa, an organism which does not involve $Me(B_{12})$ in its metabolism. Landner concluded that methylmercury was produced by an incorrect biosynthesis of methione from a mercury-homocysteine complex (Fig. 7). However, more recently it has been suggested that $Hg(SMe)_2$ forms under the conditions of the experiment[81].

Although there is considerable experimental evidence to indicate biological decomposition of methylmercury, there is little information as to the mechanism of this decomposition. Tonomura et al.[82,83] have proposed a reductive decomposition of methylmercury to metallic mercury. The mechanism involves the transfer of electrons from reduced NADP to cytochrome C via a metallic mercury-releasing enzyme, mercury becoming the terminal acceptor of the electrons in its reduction to metallic mercury. Another enzymatic decomposition of methylmercury involving an NADPH-dependent enzyme has been hypothesised by Komura and Izaki[84].

Fig. 6.

The Methionine Synthetase System

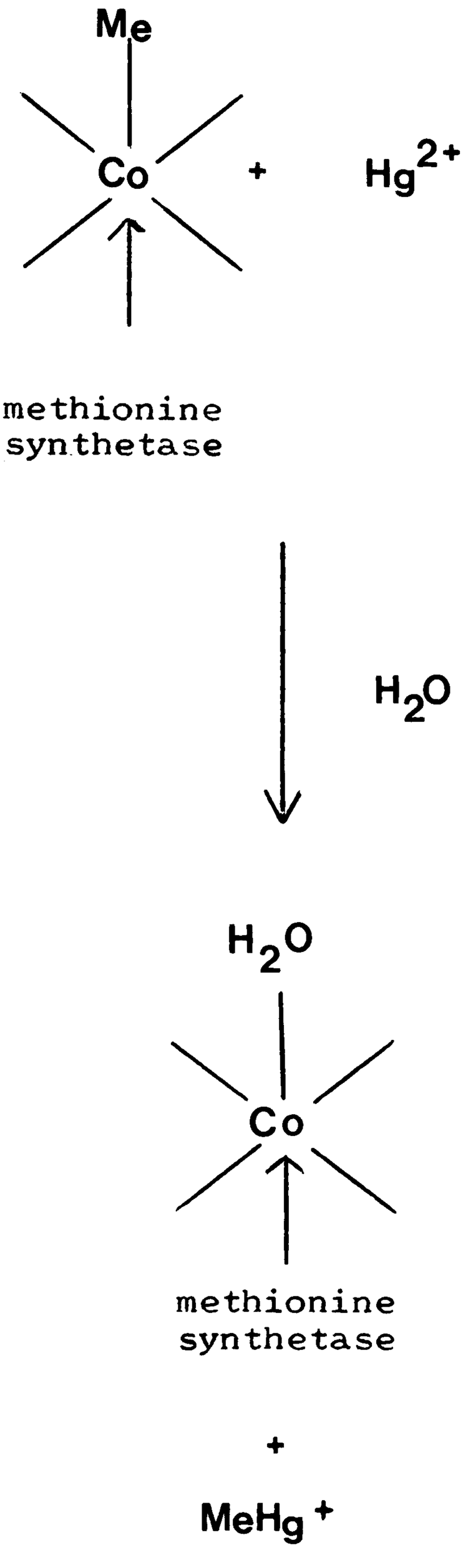
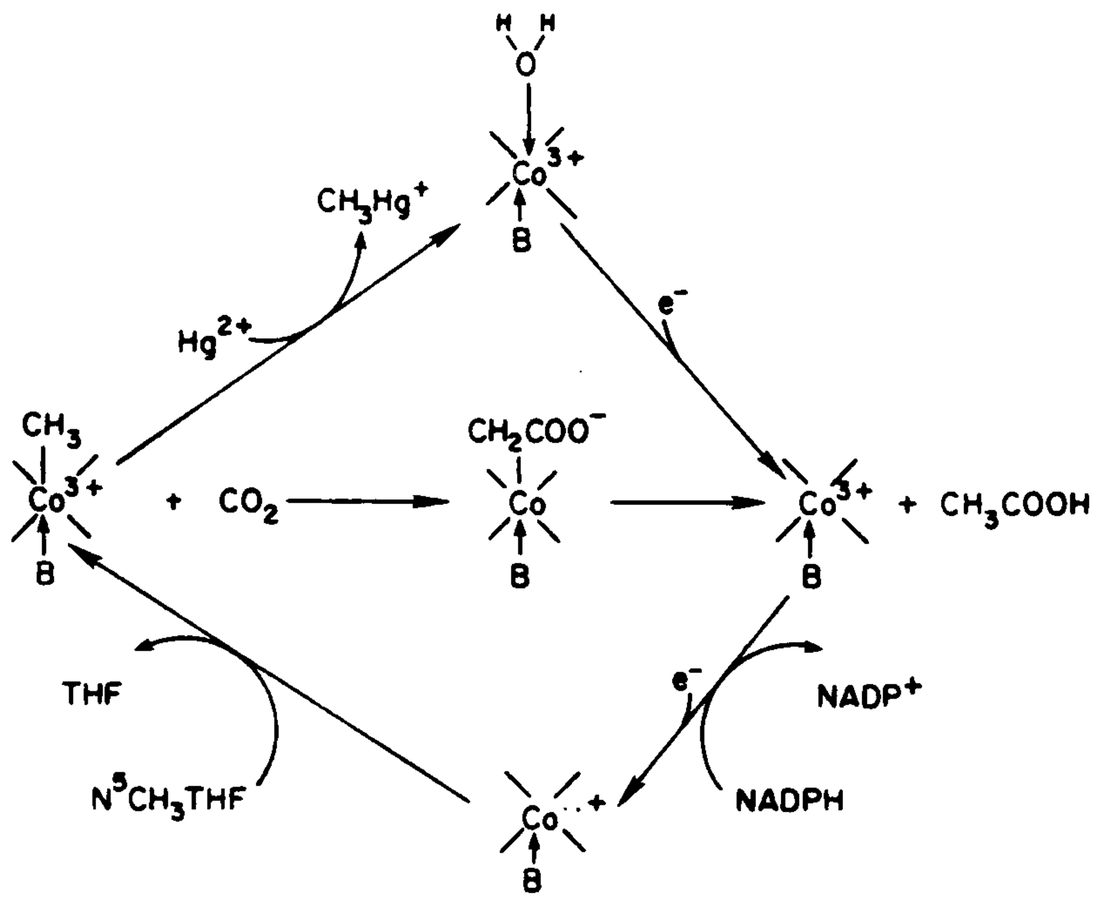
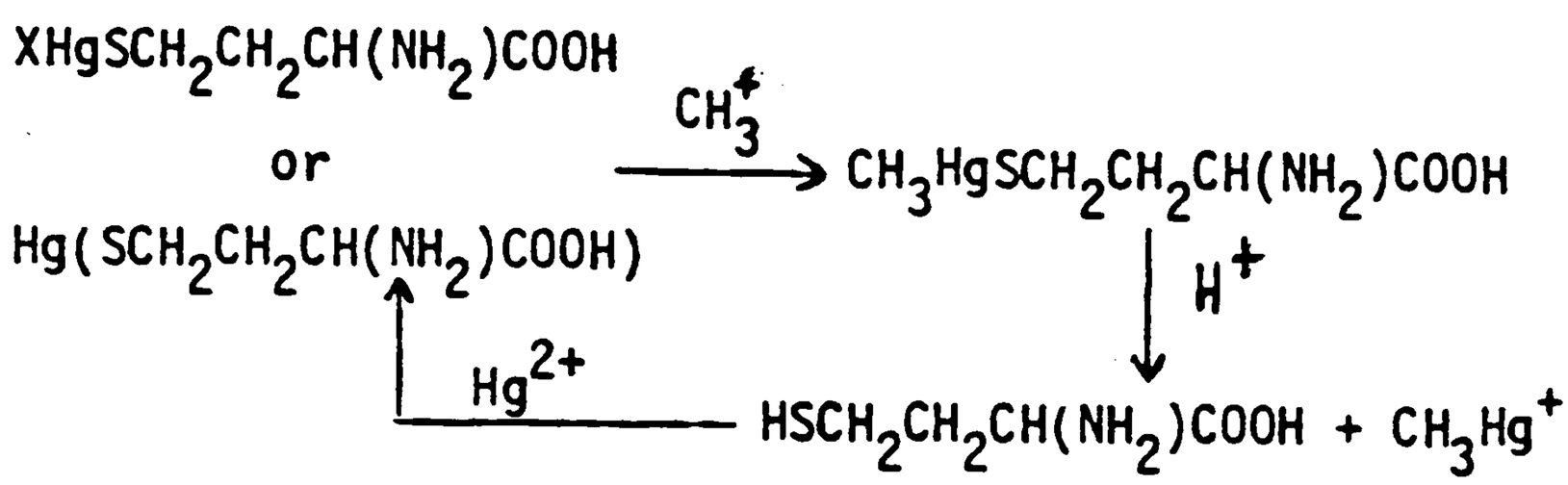


Fig. 7.



The Acetate Synthetase System



Mercury Methylation Model for Neurospora crassa, proposed by Landner[80].

Chemical Methylation and Demethylation

Chemical methylation and demethylation processes for mercury species in the environment have not been studied as extensively as the equivalent processes having biological origins. The literature on this subject is now reviewed below.

Jernelov[85] proposed trimethyllead as an effective methylating factor for mercury in the environment; this suggestion has been confirmed by other workers[78,86].

DeSimone[87] has shown that water-soluble methylsilicone compounds react with Hg (II) to give methylmercury, viz:-

$$(CH_3)_3\text{-Si-}(CH_2)_2SO_2Na + Hg^{2+} + 2OAc^- \longrightarrow (CH_3)_2\text{-}\underset{\underset{OAc}{|}}{Si}\text{-}(CH_2)_2SO_3Na + CH_3Hg^+ + OAc^-$$

Akagi et al.[88] have demonstrated photochemically induced alkylation of mercuric chloride using methanol, ethanol, acetic acid and propionic acid.

Rogers[89,90] found an alkaline extract of soil with the ability to abiotically methylate inorganic mercury. The methylating factors were not identified but were believed to be low molecular weight organic compounds associated with the fulvic acid fraction of the soil. Nagase et al.[91] extended the work of Rogers and found that alkaline extracts of river sediment and leaf mould also had the ability to methylate mercury. Methylation occurred in the absence of bacteria and was influenced by : Hg (II) concentration; humic and fulvic acid concentrations; temperature - higher temperatures enhanced methylation; pH - the optimum pH was found to be approximately 4.0. The most effective methylating factor appeared to low molecular weight (<300) compounds associated with the fulvic acid fraction of the sediment.

Another potential source of methylmercury has recently come to light. Lee et al.[92] described the occurrence of di- and monomethyl sulphate in coal fly ash at concentrations up to 1000 ug g^{-1}. These methyl sulphate compounds are very strong methylating agents, and it has been suggested that if they co-occur with Hg (0) in plumes from coal-burning industries, methylmercury may be produced[93].

A few schemes have been proposed for the abiotic decomposition of methylmercury in the environment.

Baughman et al.[94] found that methylmercuric thiol and methylmercuric sulphide ion complexes undergo photodecomposition in sunlight. However, the authors found that the low sunlight adsorption rate constants for dimethylmercury, methylmercuric ion and methylmercuric hydroxide precluded photodecomposition as a significant pathway for the degradation of these species.

Gage[95] has shown that organic mercury compounds can be decomposed in solution by ascorbate ion and soluble proteins, providing the solution is exposed to air and is free of Cu (II).

Finally, Bartlett and Craig[96] have proposed an abiotic route leading to the loss of methylmercury from sediments. The proposal is that methylmercury may react with sulphide in the sediment environment to form bis(dimethylmercury) sulphide which may then disproportionate into dimethylmercury and mercuric sulphide:

$$2MeHg^{+} + S^{2-} \longrightarrow (MeHg)_2S \longrightarrow (Me)_2Hg + HgS$$

Dimethylmercury is volatile and insoluble in water and would thus quickly be lost from the sediment environment once formed. The authors were able to demonstrate the formation of dimethylmercury from sediments which had been inoculated substantially with methylmercury and sulphide.

The limatations of this work are discussed later (Chapter 5).

Kinetic Environmental Methylation Models

Several semiempirical models have been developed to predict net methylation rates in the sediment environment and in mixed cultures of microorganisms[73,97,98,99]. One of the most detailed models was produced by Bisogni and Lawrence[98]. The authors postulated that the rate of methylmercury production in microbial systems is a function of 'available' mercuric mercury concentration and either the concentration or production rate of methyl corrinoids, enzymes or metabolic products involved in methylation. It was also postulated that the overall order of the reaction would be complex due to the fact that both nonenzymatic and enzymatic (with several different enzyme systems) methylation occurs simultaneously. The authors concluded that the overall production of methylmercury should be described by the following equation:

$$NSMR = \gamma(\beta Hg_{TOTAL})^{n} ,$$

where NSMR is the net specific methylation rate, γ is a coefficient determined by the microbial growth rate of the system, β is the ratio of free mercuric ions to total inorganic mercury and n is the pseudo-order of the reaction. From laboratory experiments with microbial reactors it was found that the pseudo-order of the methylation reaction was greater under aerobic conditions than under anearobic conditions (the average n values were 0.28 and 0.15 respectively). The significant difference in the order of the reaction was probably due to different methylation reactions predominating under anaerobic and aerobic conditions.

The NSMR model has been used with a certain amount of success to describe methylation in the sediment environment. However, the model is not generally applicable as it fails to take into consideration certain abiotic

methylation and demethylation processes. Another major impediment to the application of the model to the sediment environment derives from the fact that the affinity of sediments for methylmercury influences the rate of decomposition of the compound. Methylmercury produced in the sediment and bound by it is likely to be decomposed by sediment microorganisms. Alternatively, methylmercury desorbed from the sediment is unlikely to be decomposed before it is taken up by macrobiota in the water column, and as a consequence, net methylation in the latter situation is likely to be greater than in the former.

It is apparent that prediction of the rate of methylmercury production in a natural environment involves not only modeling biochemical and microbially mediated reactions, but also requires modelling at the same time sorption and desorption phenomena from sediments.

Chapter 4

Methylmercury Levels in the Sediment Environment

Many laboratory studies have demonstrated the formation of methylmercury in sediments that have been amended with various mercury compounds, and additionally these studies have shown that sediment parameters, such as Eh and pH, play an important role in controlling rates of production of methylmercury. However, few investigations have been carried out into relationships between actual in situ levels of methylmercury and other parameters in the natural sediment environment. Indeed, only a small amount of data for in situ methylmercury levels in sediments has been published. Some results which have been reported are presented in Table 4.

The results presented in Table 4 show that generally methylmercury accounts for only a small percentage of the total mercury present in sediments, although a high methyl/total mercury ratio has been reported for one location in the upper Rhine[53]. The limited amount of data presented in Table 4 also indicates that the methyl/total mercury ratio is independant of total mercury concentration.

In the last ten years, studies on methylmercury concentrations in U.K. sediments have been made by Morton[102] and Bartlett[103]. The present work constitutes a third and wider ranging study.

Morton investigated methylmercury levels in Mersey estuary sediments. He found that the methylmercury content of the sediments ranged from <1.0-46.9 ng g^{-1} dry weight (in 1974), and accounted on average for ~0.4% of the total mercury present. Morton also found a significant linear correlation ($P<0.01$) between methylmercury and total mercury

Location	Total Mercury ($ug\ g^{-1}$)	Methylmercury ($ng\ g^{-1}$)	Methyl/Total Mercury (%)	Reference
Monte Amiata, Italy	64 - 288	20- 40	0.03 (Mean)	52
Upper Rhine, France	0.12 - 58	6 - 659	0.06 - 72.5	53
Lower Rhine, Holland	5 - 17	10 - 110	0.2 - 1.0	54
Yessel, Holland	12	70	0.6	54
Mississippi, U.S.A.	0.08 - 0.57	< 0.02 - 0.05	0.01	100
San Fransisco Bay, U.S.A.	0.1 - 1.3	0.4 - 1.9	0.03 - 1.0	59
Wabigoon, Canada	0.35 - 66	0.3 - 44.1	0.18 (Mean)	55
Cockburn Sound, Australia	0.1 - 1.9	< 5 - 6	0.3 - 0.5	101

Table 4 : Methylmercury Levels in Sediments

levels in sediments collected during one survey of the estuary, although this correlation became less significant ($P<0.05$) when data obtained from further surveys was included in the statistical analysis. The most interesting aspect of Morton's work was his discovery that methylmercury levels in sediments can vary significantly subsequent to sampling. Morton found that sediment methylmercury concentrations increased on storage of the sediments, up to a maximum after 3-14 days, and then declined slowly over a longer time period (some of Morton's results are illustrated in Fig. 8). It was suggested that this growth and decay effect was initiated by changes in the sediment microbiology following perturbation of the sediment during sampling, although in one experiment sterilisation of the samples immediately after collection repressed only slightly the growth and decay pattern (Fig. 9). Morton postulated a two step mechanism to account for the latter observation :-

(1) Under normal conditions an equilibrium is established between methylation and demethylation processes. The methylation process involves reaction of a biologically produced methylating agent with inorganic mercury, the agent being produced at a rate which ensures that a continuous supply of the agent is present in the sediment.

(2) Following sterilisation of the sediment, biological demethylation processes are su pressed and inorganic mercury reacts with the existing methylating factor, resulting in methylmercury production, until the supply of methylating agent is depleted. On depletion of the methylating agent, loss mechanisms for methylmercury predominate resulting in a fall in methylmercury levels.

Bartlett extended Morton's work and suggested that the growth and decay effect was initiated by positive changes in the redox potential of the sediments which followed

Fig. 8.

Growth and Decay of Methmercury Levels in a Mersey Sediment During Storage

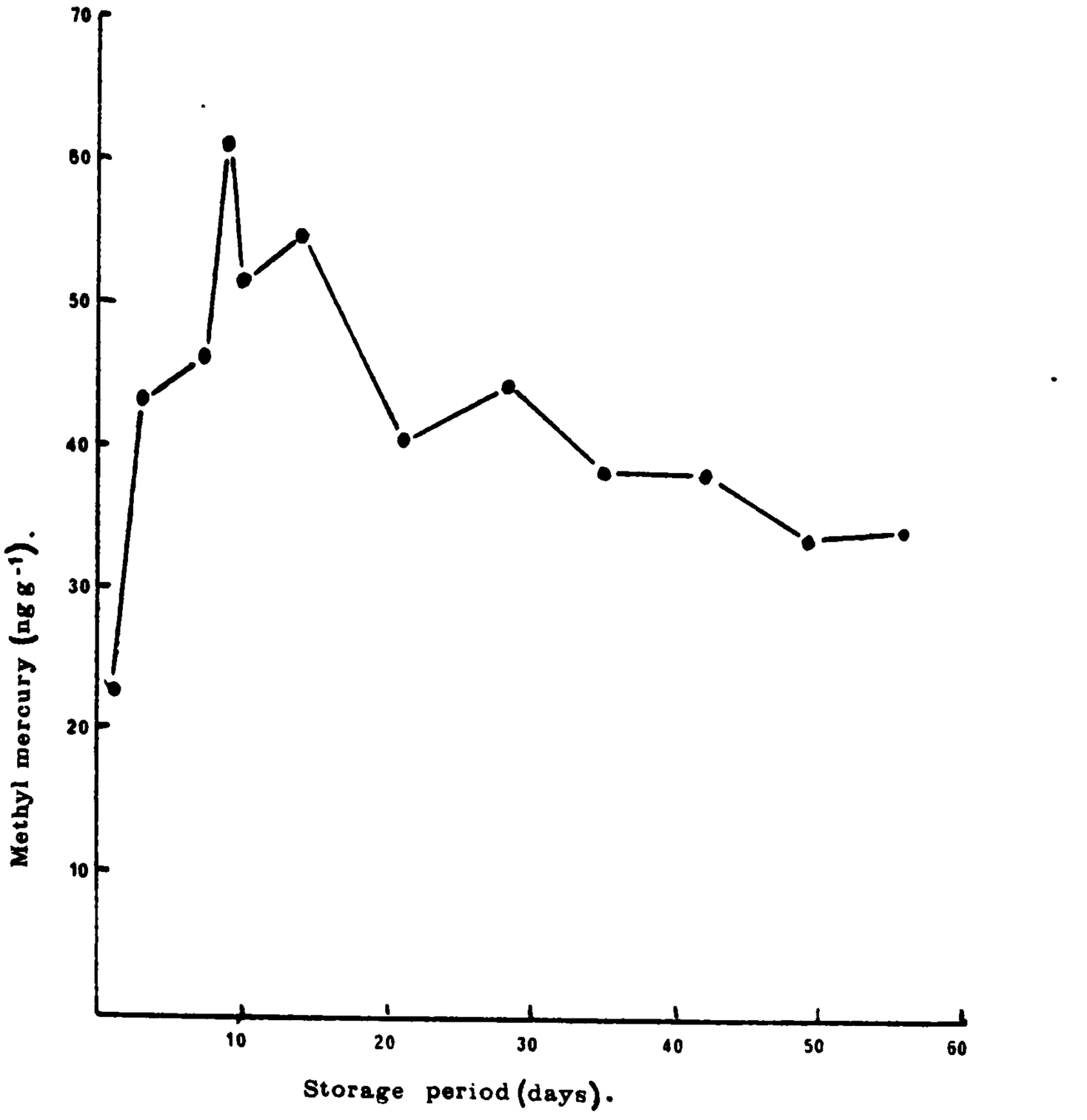
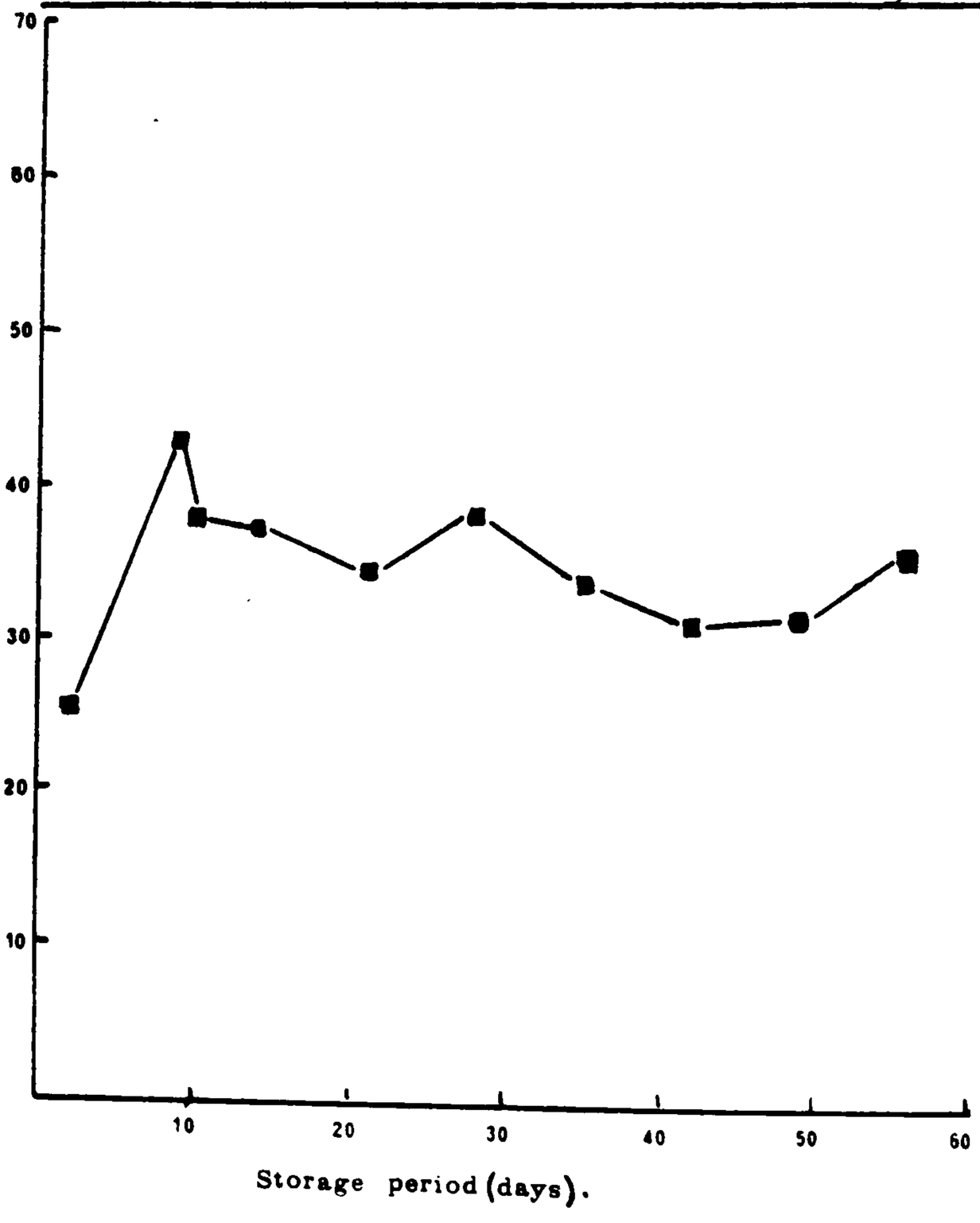


Fig. 9.

Growth and Decay of Methylmercury Levels in a sterilised Sediment During Storage



incorporation of air into the sediments during sampling. Two possible mechanisms were postulated to account for the phenomenon:-

(1) The oxygen input into the sediment stimulated aerobic bacteria into faster enzyme and methylating agent production, resulting in faster methylation of the inorganic mercury present. When anaerobic conditions returned, the production rate slowed resulting in a fall in methylmercury concentration.

(2) Oxygen input on sampling resulted in a fall in sulphide levels in the sediment, and hence in a reduced rate of loss of methylmercury via dismutation through the sulphide route, viz:-

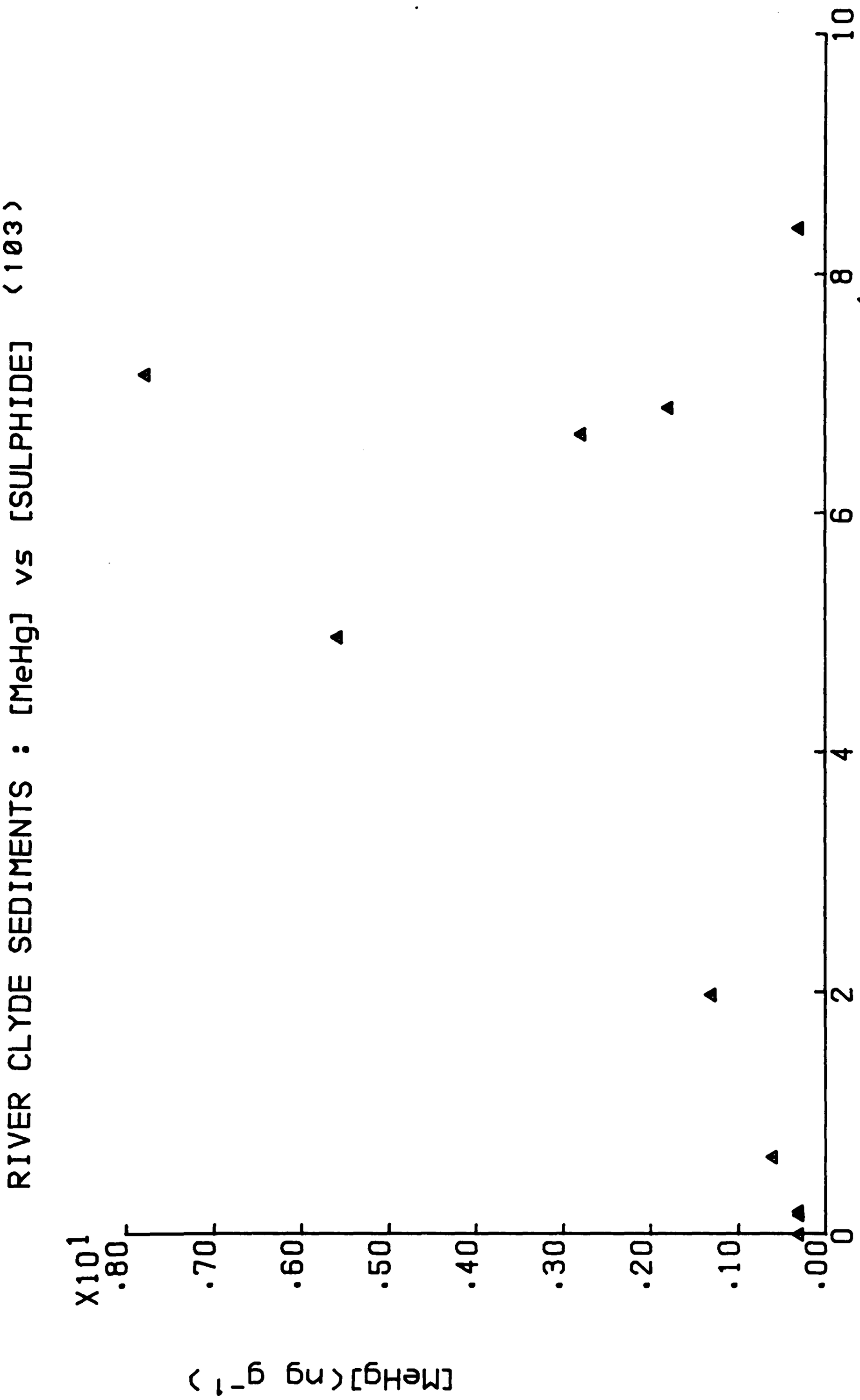
$$2MeHg^{+} + S^{2-} \longrightarrow (MeHg)_2S \longrightarrow Me_2Hg + HgS$$

Interestingly, Bartlett found that the growth and decay effect occurred only in sediments collected from certain locations in the Mersey, and moreover, this effect was observed to become less pronounced during the course of study. For those sediments which did not exhibit a growth and decay pattern, changes in methylmercury levels during storage appeared to mirror changes in sulphide levels. Bartlett analysed four sediment samples for methylmercury and found a high correlation ($P<0.001$) between these two parameters.

Bartlett also carried out an extensive survey of Mersey estuary sediments, and found high correlations between (1) in situ methylmercury and total mercury levels ($P<0.001$), and (2) methylmercury levels and silt content ($P<0.001$). A poorer, although significant, correlation also was found between methylmercury levels and organic content ($P<0.01$). On average the methylmercury content of the sediments was found to account for 0.46 per cent of the total mercury present.

Bartlett also reported results from surveys of the River Clyde. The results showed that the methyl/total mercury ratio for Clyde sediments was generally similar to that found in Mersey sediments. However, Bartlett found no correlation between methylmercury, total mercury, organic carbon content or Eh value in Clyde sediments. Interestingly, a plot of methylmercury and sulphide concentrations in Clyde sediments (Fig. 10) indicated a direct relationship between methylmercury and sulphide concentrations up to concentrations of ~5.8 mg g^{-1} sulphide. At this point methylmercury reached a maximum concentration, and then appeared to decline with further increase in sulphide. However, Bartlett was unable to confirm this relationship between the two parameters with the limited amount of data available.

Fig. 10.


RIVER CLYDE SEDIMENTS : [MeHg] vs [SULPHIDE] (103)
[MeHg](ng g⁻¹)
X10¹
.80
.70
.60
.50
.40
.30
.20
.10
.00
0
2
4
6
8
10
[SULPHIDE](mg g⁻¹)

Chapter 5

Aims and Objectives of this Work

Laboratory studies have shown that the degree of anoxicity of an environment is an important factor in controlling both rates of formation and decomposition of methylmercury. However, there is a scarcity of information relating in situ levels of methylmercury in natural sediments with their degree of anoxicity. One of the aims of this project was to obtain such information, measuring the degree of anoxicity of sediments both in terms of Eh value (which provides a measurement of the ratio of oxidised to reduced species in a system) and sulphide content. The possibility of relationships existing between methylmercury, total mercury and organic carbon content of sediments was also to be explored. This would allow an assessment of the factors controlling methylmercury production.

The principal aim of the project was to investigate the relationship between in situ levels of methylmercury and sulphide in river and estuarine sediments. Three aspects of Bartlett's work had indicated that these two parameters may be related:-

(1) Laboratory experiments had shown that methylmercury reacts with sulphide to form bis-dimethylmercury sulphide, and this compound may dismutate under environmental conditions to volatile dimethylmercury and inert mercuric sulphide, thereby contributing to mercury transport in the general environment. Bartlett had demonstrated the evolution of dimethylmercury from sediments which had been amended in the laboratory with high concentrations of methylmercury (100 ug g^{-1}) and saturated with hydrogen sulphide. However, these were extreme conditions beyond those met in the natural environment.

(2) Limited data obtained from the analysis of River Clyde

sediments had suggested that loss of methylmercury through the sulphide route may become dominant at sulphide concentrations greater than $\sim$5.8 mg g^{-1}. However, this suggestion had been based on 10 data points collected from one location and the general validity of the relationship had not been established.

(3) The results of incubation experiments with natural sediments had suggested the existence of a linear relationship between methylmercury and sulphide levels in sediments with low sulphide contents.

For this work a number of rivers and estuaries were selected for survey in order to investigate the relationship between sediment methylmercury and sulphide levels. The sediments of these water systems were known from previous work to contain different levels of total mercury and sulphide, and were as follows: The Carron - high mercury, high sulphide; The Clyde - low mercury, high sulphide; The Mersey - high mercury, low sulphide; and various estuaries in S.W. England - low mercury, low sulphide.

However, before the work could be undertaken, it was necessary to accomplish two objectives. Firstly, in view of Morton's discovery of the time-dependency of sediment methylmercury levels, it was felt necessary to develop a sampling and storage procedure capable of "preserving" in situ levels of methylmercury in sediments. Secondly, although Morton and Bartlett had developed satisfactory methods for the determination of methylmercury and total mercury in sediments, Bartlett's method of sulphide determination was known to be subject to strong interferences from some oxidation products of sulphide, and hence inaccurate; the second objective was therefore to develop a precise and accurate method of sulphide determination. Additionally, it was hoped to further develop and improve the methods for methylmercury and total mercury determination during the course of the project.

Finally, it was hoped to establish conclusively the

importance of the sulphide route in leading to loss of methylmercury by demonstrating the evolution of dimethylmercury fron natural sediments with high sulphide contents not amended by methylmercury. This had not been demonstrated previously.

A further aim of the project was to synthesise model compounds for mercury as it is likely to exist coordinated in the sediment environment, and to investigate the capacity of these compounds to undergo methylation in river sediments. Possible reactions of these compounds with the natural methylating agent, $Me(B_{12})$, were also to be explored. Additionally, the possibility of mercury and its compounds reacting with other natural methylating agents, e.g. iodomethane and betaine, was to be investigated. Positive results for these experiments would indicate additional routes, previously unreported, leading to formation of methylmercury in the natural environment. Finally, the relative importance of biological and abiotic processes in producing environmental methylmercury levels was to be investigated.

In order to achieve these ends the following methodology was adopted - under three headings : analytical development, environmental work and synthetic work.

Analytical Development

It was proposed to :-

(1) Refine and improve the methods for the determination of methylmercury and total mercury in sediments developed by Morton and Bartlett.

(2) Review the literature on the determination of sulphide in environmental samples, and then develop a quick, precise and accurate method for the determination of sulphide in sediments.

(3) Devise a sampling and storage procedure capable of preserving in situ methylmercury levels of sediments.

Environmental Work

It was proposed to :-

(4) Investigate the relationship between methylmercury and sulphide levels in sediments.
(5) Investigate the relationship between methylmercury levels and other sediment parameters, e.g. Eh, total mercury and organic carbon content.

Synthetic Work

It was proposed to : -

(6) Demonstrate the evolution of dimethylmercury from natural sediments with high sulphide contents.
(7) Carry out differential incubation experiments with several model mercury compounds in river sediments, and thus to investigate the implications of different types of coordination to mercury for methylmercury production.
(8) Investigate the ability of mercury and its compounds to react with natural methylating agents, and to elucidate possible new routes leading to the formation of methylmercury in the environment.
(9) Investigate the relative importance of biological and abiotic processes in producing environmental methylmercury levels.

SECTION 2

ANALYTICAL DEVELOPMENT

Chapter 6

The Determination of Sulphide in Sediments

Introduction and Literature Review

Sulphide is present in river waters and sediments as a result of the decomposition of organic matter and natural ore bodies. Microorganisms play an important role in both the production and removal of sulphide from the aquatic environment: sulphide is produced when organic matter is decomposed by anaerobic bacteria, and aerobic bacteria can remove sulphide through oxidation. Sulphide is also introduced into the aquatic environment as a pollutant from various industrial plants, most notably: sewage works, paper mills, tanneries and other chemical industries.

Sulphide levels in sediments are of interest in this project as sulphide can precipitate and immobilise mercury as mercuric sulphide (HgS) and may thus play an important role in the formation of a mercury sink in the environment. However, it is known that alkali sulphides can dissolve HgS to form complex ions of the type HgS_2^{2-} (or possibly $[Hg(SH)_4]^{2-}$)[104], although it is uncertain whether these reactions appreciably influence the mobility of mercury in natural waters. Also, the production of methylmercury from HgS in aerobic, organic sediments has been demonstrated[62], although the yields and rates of production are small. Finally, hydrogen sulphide has been shown to be capable of mobilising methylmercury via an organomercury derivative which may dismutate into HgS and volatile $(Me_2)Hg$, viz:-

$$2MeHg^{+} + S^{2-} \longrightarrow (MeHg)_2S \longrightarrow HgS + (Me)_2Hg \quad [105]$$

The most popular methods for the determination of sulphide in sediments can be grouped into three categories: iodometric, colorimetric and electrochemically by the use of sulphide specific electrodes. In recent years interest

also has been shown in the technique of gas-phase molecular absorption spectrometry.

Iodometric Methods

Workers in the fields of pollution control and public health frequently use titrimetric methods in which sulphide is first oxidised by an excess of standard iodine solution following which unreacted iodine is back titrated with standard thiosulphate. The method is not specific if iodine is added to the sample directly,as a number of other species present in sediments reduce iodine, most notably sulphite and thiosulphate[106]. However, some workers have used this approach to obtain approximate values for the concentration of sulphide in sediments. To make the method specific, acidification of the sample under an inert atmosphere is required as a first step. The evolved hydrogen sulphide is flushed into a trapping solution - e.g. zinc acetate, where the sulphide is precipitated as ZnS - which is mixed subsequently with standard iodine solution, acidified and back titrated with thiosulphate. A number of experimental procedures for this method have been published, e.g. by Allen and Grimshaw[107]. There are a number of drawbacks to this method, e.g. it is slow, requires much glassware, extraction efficiencies are variable and it is unsuitable for handling large numbers of samples.

Munson[108] has described an iodometric method which is specific but which also overcomes the disadvantages listed above. In this method, samples are acidified in a digestion vessel and the evolved hydrogen sulphide is precipitated onto a paper wick impregnated with zinc acetate, the paper wick being suspended in the digestion vessel. The wick is added subsequently to standard iodine solution, which is then acidified and the excess iodine back titrated with thiosulphate. However, poor trapping efficiencies are obtained with samples containing more than 350 ug of sulphide, and the method is suitable only for samples containing

relatively low sulphide concentrations.

Colorimetric Methods

Colorimetric methods are used commonly to determine sulphide concentrations in non-turbid solutions, and generally they are more suitable than iodometric methods for determining low levels of sulphide (ppb range). Most published methods are based on the reaction which takes place, under suitable conditions, between para-aminodimethylaniline, ferric chloride and sulphide ion, resulting in the formation of methylene blue ($C_{16}H_{18}N_3SCl$). Ammonium phosphate is added prior to colour comparisons with standards to remove colour caused by the presence of ferric ion. The method is not suitable for direct analyses of sediment samples as suspended material and turbidity interfere, but even with clear waters it is subject to interferences: sulphite and thiosulphate interfere if their concentrations are greater than 40 mg dm^{-3} [109], nitrite interferes at 0.5 mg dm^{-3} [109] and mercury and copper are also reported as interferents[110]. The method can be made specific for sulphide by prior treatment of the sample with acid to liberate hydrogen sulphide, but this incurs some of the disadvantages mentioned for the iodometric method. Some workers have reported that greater sensitivity is achieved if ethylene blue is synthesised rather than methylene blue[111,112,113]. A similar colorimetric method depending on the formation of Lauths Violet is also well established[114].

Darcel and Ali[115] have reported a colorimetric method based on the blue colour which is developed when hydrogen sulphide is dissolved in ammonium molybdate solution. To speed up the time of analysis the authors used steam to displace hydrogen sulphide from samples acidified with hydrochloric acid; however, under these conditions thiosulphate and thionate will yield some hydrogen sulphide.

Giammateo[116] has described a colorimetric method in

which a buffered aqueous suspension of sediment is mixed with a carbon-tetrachloride solution of silver dithizonate. Sulphide strips silver from the dithizonate complex producing free dithizone in the carbon tetrachloride layer. An aliquot of the organic phase is withdrawn, centrifuged and the absorbance of dithizone measured at 618 nm, the magnitude of the absorbance being dependant upon the concentration of dithizone in the organic phase which in turn is dependant upon the amount of sulphide in the sample. By altering the pH of the buffered suspension it is possible to distinguish between free and bound sulphide. This method is quicker than most colorimetric methods and it is relatively immune to interferents; however, experimental conditions need to be carefully controlled in order to obtain precise results.

A major disadvantage of all colorimetric methods is the necessity to prepare standard sulphide solutions which, because of the ease and speed of their oxidation by atmospheric oxygen, are difficult to prepare and often imprecise.

Electrode Methods

In recent years the use of sulphide specific electrodes has become important. Most of the sulphide electrodes now available commercially are solid-state membrane electrodes employing a disc of crystalline silver sulphide[117]. The disc acts as a solid-state ion-exchange membrane specific for silver and sulphide ions and allows these ions to impose a potential on an internal electrode contained within the main electrode body. The magnitude of the imposed potential depends upon the concentration, or more precisely, the activity of the ions in the sample. It should be noted that these electrodes respond to divalent sulphide ions only (and Ag^+); hydrosulphide ions and hydrogen sulphide produce no response. The relative ratios of divalent sulphide ion, hydrosulphide ion and hydrogen sulphide in aqueous samples vary with pH and temperature, and the

response of the electrode is thus governed by the pH and temperature of the sample. Also, the response of the electrode depends upon the ionic strength of the sample as the electrode responds to activity, not concentration, of ions.

Berner[118] and Whitfield [119] have used sulphide electrodes in conjunction with pH electrodes to measure sulphide concentrations in sediment pore waters directly. A drawback of this method is the necessity to prepare sulphide standard solutions to calibrate the sulphide electrode.

Another electrode method has been developed by Green and Schnitker[120] who determined sulphide levels in sediments by potentiometric titrations using a commercial electrode. In this method sediment samples are dispersed in a sulphide anti-oxidant buffer (SAOB - a solution of potassium hydroxide and ascorbic acid) and titrated with standard cadmium nitrate solution, the end point being detected potentiometrically by the electrode. The SAOB has two functions: it prevents oxidation of sulphide by reducing any oxygen diffusing into the solution and by poising the redox potential at a value at which sulphide oxidation is unfavourable; and it converts hydrogen sulphide and hydrosulphide ion into the divalent species detected by the electrode. Frant and Ross[121] developed the first SAOB but improved formulations have been suggested since[122]. Elemental sulphur, which is partially converted into sulphide by the extremely alkaline SAOB, is an interferent in this method.

<u>Gas-phase Molecular Absorption Spectrometry (G.M.A.S.)</u>

Cresser[123] and Syty[124] have shown that sulphide concentrations can be determined by measurement of the U.V. absorption of hydrogen sulphide evolved upon acidification of aqueous samples. The method makes use of an atomic absorption spectrometer modified for cold vapour analysis. Hydrogen sulphide evolved from the sample is flushed into

a flow-through cell situated in the light path of a deuterium lamp, and the absorbance at 200 nm is recorded; the sulphide concentration is then determined by refering the value of absorbance obtained to a calibration graph. Sediments cannot be analysed directly by this method as the difference in matrix composition between the aqueous standards and sediment samples results in different rates of release of hydrogen sulphide. However, Cresser[123] has suggested that water-soluble sulphide in sediments may be extracted into an SAOB solution which may then be analysed in the normal way after centrifuging to remove suspended matter. Sediments analysed in this way gave higher results than those obtained by standard ion-selective electrode techniques[123]; this difference could have been due to the presence of colloidal iron sulphide in the SAOB extract. Sulphite and nitrite have been shown to be interferents for this method[123,124], and the need to prepare sulphide standard solutions also constitutes a major drawback.

Other methods for the determination of sulphide in sediments have been published but they are, by comparison, little used. These methods involve the use of gas chromatography[125], gravimetry[126], manometry[126], polarography[127], indirect atomic absorption spectrometry[128] and Draeger tubes[129].

It should be noted that methods involving acid treatment of samples measure acid-soluble as well as water-soluble sulphide; whereas methods involving no acid treatment, e.g. electrode techniques, determine water-soluble sulphide only. Attempts have been made to measure water-soluble sulphide in sediments by applying acid-treatment techniques to the interstitial water of sediments. However, removal of interstitial water by squeezing[130] has been shown by Berner[131] and Bray et al[132] to result in loss of sulphide; although Kalil and Goldhaber[133] seem to have

overcome this problem by excluding air.

Sampling

It is believed generally that the low stability of sulphide necessitates special precautions to be taken when samples are collected and transported back to the laboratory for analysis. Considerable loss of sulphide from sediments may occur during sampling and transportation, due mainly to volatilisation of hydrogen sulphide and oxidation by air. Samples to be analysed by acid-digestion techniques can be "preserved" with zinc acetate, and samples to be analysed for water-soluble sulphide should be transported in air-tight containers from which oxygen has been excluded. In either case, the time between collection and analysis of samples should be kept to a minimum as microbiological activity can alter sulphide levels during storage. Sampling and storage procedures are discussed further in Chapter 11.

Introduction to Experimental Work

One of the objectives of this project was to develop a method for sulphide determination that is accurate, precise and quick. To this end a comparison of some of the methods described above was undertaken. Also, because the techniques measure different sulphide species, it was felt important to develop a comparison between them in order to extrapolate between different methods and measurements and to make proper comparison with previously published work.

Experimental Work

Digested sewage sludge was obtained from Wanlip Sewage Treatment Plant, Leicestershire (Severn Trent Water Authority). The sludge was allowed to settle and the supernatent liquid decanted from it. The sulphide content

of replicate samples of the remaining solids was determined by four methods: (1) direct iodometric titration, (2) volatilisation of hydrogen sulphide followed by iodometric titration (indirect iodometric), (3) gas-phase molecular absorption spectrometry (G.M.A.S.) and (4) potentiometric titration.

Individual samples were taken from an homogenised 1 Kg sample of sewage sludge solids. The samples were weighed quickly to avoid changes in sulphide levels due to oxidation and microbiological activity. Samples for analysis by methods (1) and (2) were analysed immediately after being weighed; samples for analysis by methods (3) and (4) were preserved in SAOB solution and analysed as soon as possible to avoid any difference caused by deterioration of the samples.

The dry weight of the sewage sludge solids was determined by drying 10g of sample at 110°C to constant weight. Water content ranged from 54% to 56%.

(1) Direct Iodometric

Reagents:-

Iodine (A.R.) 0.005 mol dm^{-3} aqueous

Sodium thiosulphate (A.R.) 0.01 mol dm^{-3} aqueous

Method

Up to 1g of wet sample was weighed into a 10cm^3 centrifuge tube to which 10cm^3 of standard iodine solution was then added. The tube was stoppered and shaken for two minutes. The mixture was left for 1 hour and centrifuged. After centrifuging, 5 cm^3 of solution containing excess iodine was removed and titrated with standard thiosulphate solution.

Calculation of Sulphide Concentration in Sample

One mole of iodine reacts with two moles of thiosulphate according to the equation:

$$I_2 + 2S_2O_3^{2-} \longrightarrow 2I^- + S_4O_6^{2-}$$

The amount of excess iodine in the solution is therefore equal to titre/1000 x 0.01/2 moles. The amount of iodine originally present in 5 cm^3 of iodine solution is equal to 5/1000 x 0.005 moles. Therefore, the amount of iodine consumed is equal to:

$$\frac{5}{1000} \times 0.005 - \frac{titre}{1000} \times \frac{0.01}{2} \quad \text{moles}$$

The number of moles of iodine consumed in 10 cm^3 of solution will be double this amount, and this will also be equal to the number of moles of sulphide in the sample, as one mole of iodine reacts with one mole of sulphide according to the equation:

$$2S^{2-} + 2I_2 \longrightarrow 4I^- + S_2$$

Incorporating the percentage dry weight of the sample into the equation and multiplying the number of moles of iodine consumed by 32,000, the concentration of sulphide in the sample, in units of mg g^{-1} dry weight, is equal to:

$$\left(\frac{5}{1000} \times 0.005 - \frac{titre}{1000} \times \frac{0.01}{2} \right) \times 2 \times \frac{1}{\text{wt. of sample}} \times \frac{100}{\% \text{ dry wt.}} \times 32000$$

Five portions of sewage sludge were analysed as described. The following results were obtained:-

Sample No.	Sulphide conc. (mg g^{-1})
1	2.10
2	1.84
3	1.94
4	2.03
5	1.94
Average	1.97
Stand. dev.	0.10
Coeff. var.	5.0 %

(2) Indirect Iodometric

Reagents:-

Iodine (A.R.) 0.005 mol dm^{-3} aqueous

Sodium thiosulphate (A.R.) 0.01 mol dm^{-3} aqueous

Zinc acetate 2 mol dm^{-3} aqueous

Sulphuric acid 50% V/V

Hydrochloric acid conc.

Method

The apparatus used for the evolution and trapping of H_2S is illustrated in Fig. 11. About 5g of wet sample were weighed into the digestion vessel, 50 cm^3 of zinc acetate solution was introduced into the conical flask and the apparatus connected as shown. Nitrogen was passed through the vessel to displace air, and the sample then acidified by injecting 20 cm^3 of sulphuric acid through the injection port. The digestion vessel was heated to 100°C by the hot plate and a flow of nitrogen maintained through the system for 1 hour, until all the evolved H_2S had been carried over. The bubbler tube was then removed from the conical flask and 20 cm^3 of iodine solution, followed by 5 cm^3 of concentrated hydrochloric acid, were added to the flask. The flask was stoppered and shaken for about 10 seconds following which the excess iodine was titrated immediately with standard thiosulphate solution.

Calculation of Sulphide Concentration in Sample

The amount of excess iodine in the solution is equal to titre/1000 x 0.01/2 (see page 47).

The amount of iodine originally present in the solution is equal to 20/1000 x 0.005 moles. The number of moles of iodine consumed, and hence the number of moles of sulphide in the sample (see page 47) is therefore equal to:

$$\frac{20}{1000} \times 0.005 - \frac{\text{titre}}{1000} \times \frac{0.01}{2} \quad \text{moles}$$

Fig. 11.

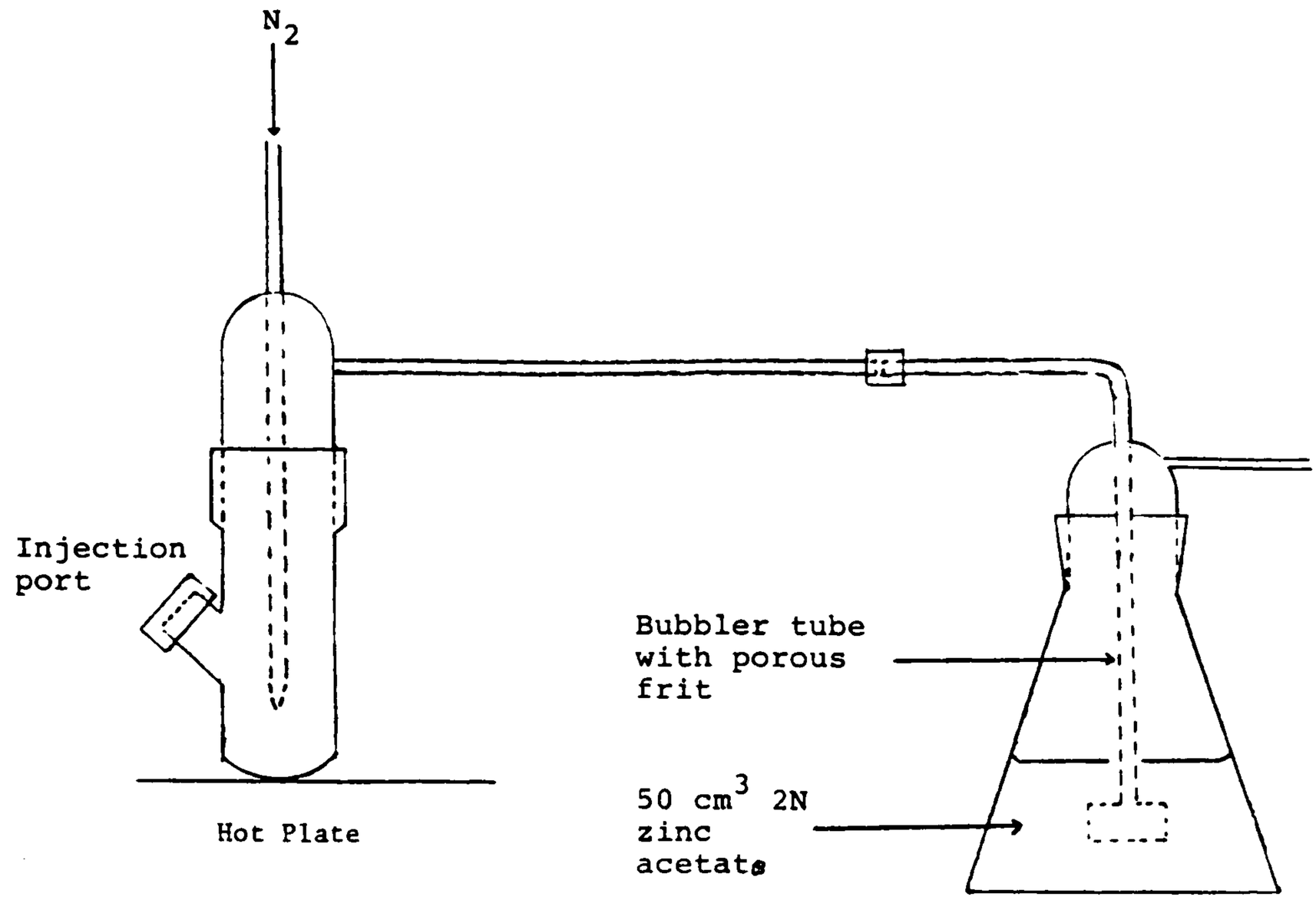
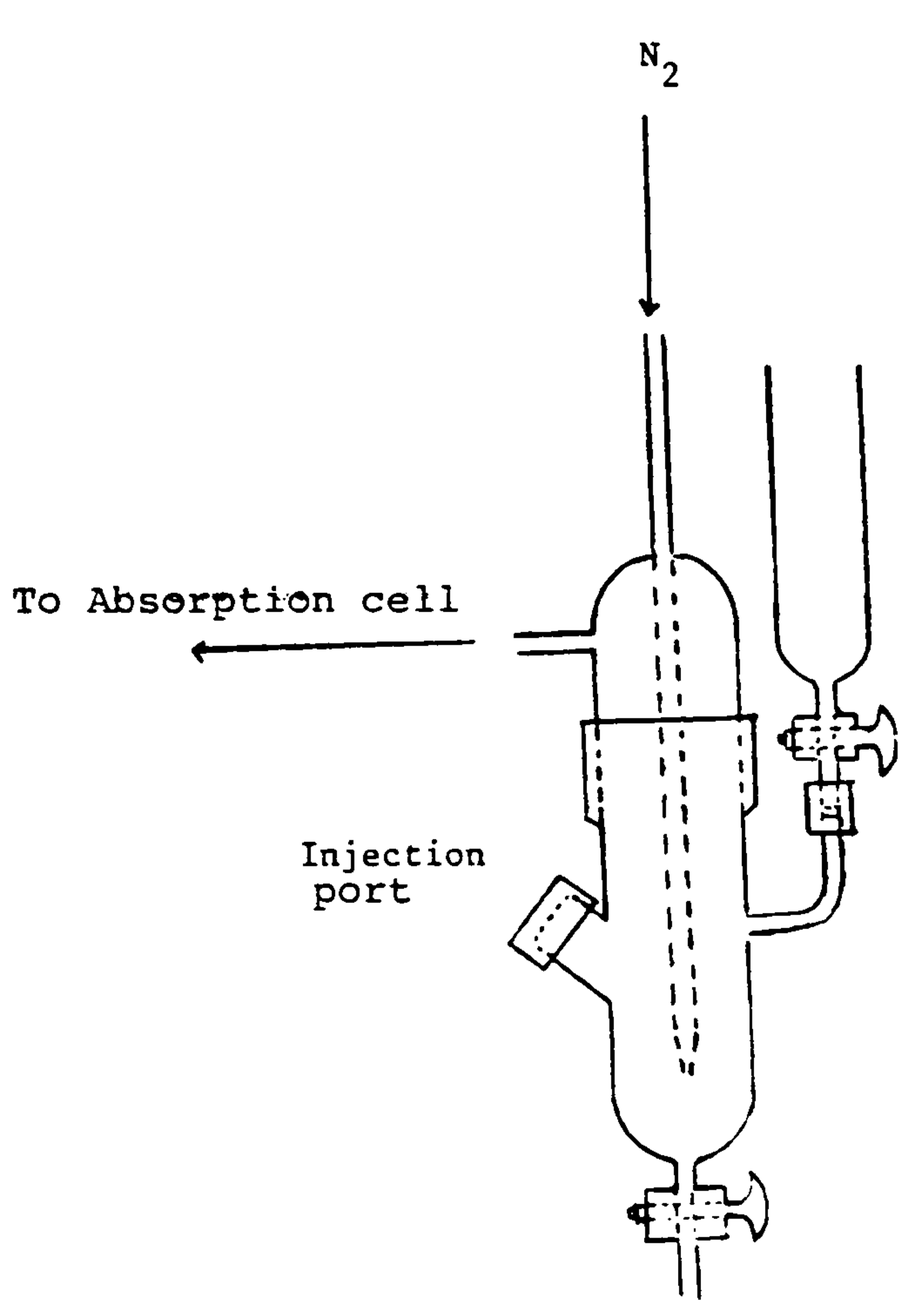

N_2
Injection port
Hot Plate
Bubbler tube with porous frit
50 cm^3 2N zinc acetate

Fig. 12.


N_2
To Absorption cell
Injection port

Incorporating the percentage dry weight of the sample into the equation and multiplying the number of moles of iodine consumed by 32000, the concentration of sulphide in the sample, in units of mg g^{-1} dry weight, is equal to:

$$\left(\frac{20}{1000} \times 0.005 - \frac{\text{titre}}{1000} \times 0.01 \right) \times \frac{1}{\text{wt. of sample}} \times \frac{100}{\% \text{ dry wt.}} \times 32000$$

Five portions of sewage sludge, identical with those analysed by Method 1, were analysed as described, and the following results were obtained:-

Sample No.	Sulphide conc. (mg g^{-1})
1	0.78
2	0.75
3	0.83
4	0.95
5	0.82
Average	0.83
Stand. dev.	0.08
Coeff. var.	9.2 %

Experiments were performed later to assess the recovery of sulphide "spikes" from sewage sludge. A standardised aqueous solution of sulphide was used as the spike. The results are shown below:-

Weight of sample (g)	Sulphide added (mg)	Sulphide found (mg)	Sulphide recovered (mg)
5.21	0	0.45	0
5.31	0	0.40	0
5.05	0	0.42	0
5.18	0.62	0.98	0.56
5.25	0.62	0.99	0.56
5.08	0.62	1.01	0.60

Mean recovery of the three spiked analyses = 0.57 mg, i.e. 92% of the spike.

(3) G.M.A.S.

Reagents:-

Hydrochloric acid 50% v/v

SAOB : Dissolve 560g of potassium hydroxide in 800cm^3 of distilled water, allow to cool and add 17.6g of ascorbic acid. Make up solution volume to 1 dm^3 with distilled water and store the solution under a blanket of nitrogen in an air-tight polythene bottle.

Sulphide standard solutions : A sulphide master standard of nominal concentration 1000 mg dm^{-3} was prepared by dissolving 0.75 g of sodium sulphide ($Na_2S.9H_2O$) in 100 cm^3 of freshly prepared distilled water. Standardisation of this solution by iodometric titration revealed the true sulphide concentration of the solution to be 940 mg dm^{-3}. Working standards were prepared by pipetting 10, 7.5, 5.0, 2.5 and 1.0 cm^3 portions of the stock solution into 100 cm^3 vol. flasks containing 25 cm^3 SAOB. The volumes were made up to the mark with distilled water producing solutions of sulphide concentration 94.0, 70.5, 47.0, 23.5 and 9.4 mg dm^{-3} respectively.

Method

An Instrumentation Laboratory Inc. Model 151 atomic absorption spectrometer modified for non-flame cold vapour analysis was used to make absorption measurements of evolved H_2S. The gas was led into a 10 cm long quartz-windowed flow-through absorption tube situated in the light path of a deuterium hollow cathode lamp. Absorption measurements were made at 200 nm with a slit setting allowing a bandwidth of 2 nm and were recorded as peak heights on a chart recorder. The digestion vessel used for the evolution of H_2S essentially is that described by Syty[124] and is illustrated in Fig. 12. Nitrogen was used to sweep H_2S out of the vessel into the absorption tube; the

nitrogen flow rate was regulated by a GEC Marconi Rotameter.

Preparation of Calibration Graph

From the burette, 20 cm^3 of hydrochloric acid was introduced into the digestion vessel (see Fig. 12). The flow of the carrier gas was set at 1.5 dm^3 min^{-1} and a baseline established on the recorder. A 1 cm^3 aliquot of one of the sulphide standards was injected through the rubber septum covering the injection port by means of a syringe. As soon as the peak maximum of the absorption signal had been recorded, the vessel was emptied via the stopcock and the vessel refilled with a further 20 cm^3 of hydrochloric acid. When the recorder pen had returned to the baseline, another 1 cm^3 aliquot of the sulphide standard was injected into the vessel and the peak maximum of the absorption signal recorded.

Five replicate injections for each of the sulphide working standards were made. The results are presented below:-

Sulphide Std.	Av. peak height	Stand. dev.	Coeff. var.
94.0 mg g^{-1}	78 mm	3.59 mm	3.9 %
70.5 " "	57 mm	2.39 mm	4.2 %
47.0 " "	39 mm	1.60 mm	4.1 %
23.5 " "	17 mm	1.51 mm	8.9 %
9.4 " "	8 mm	1.10 mm	13.8 %

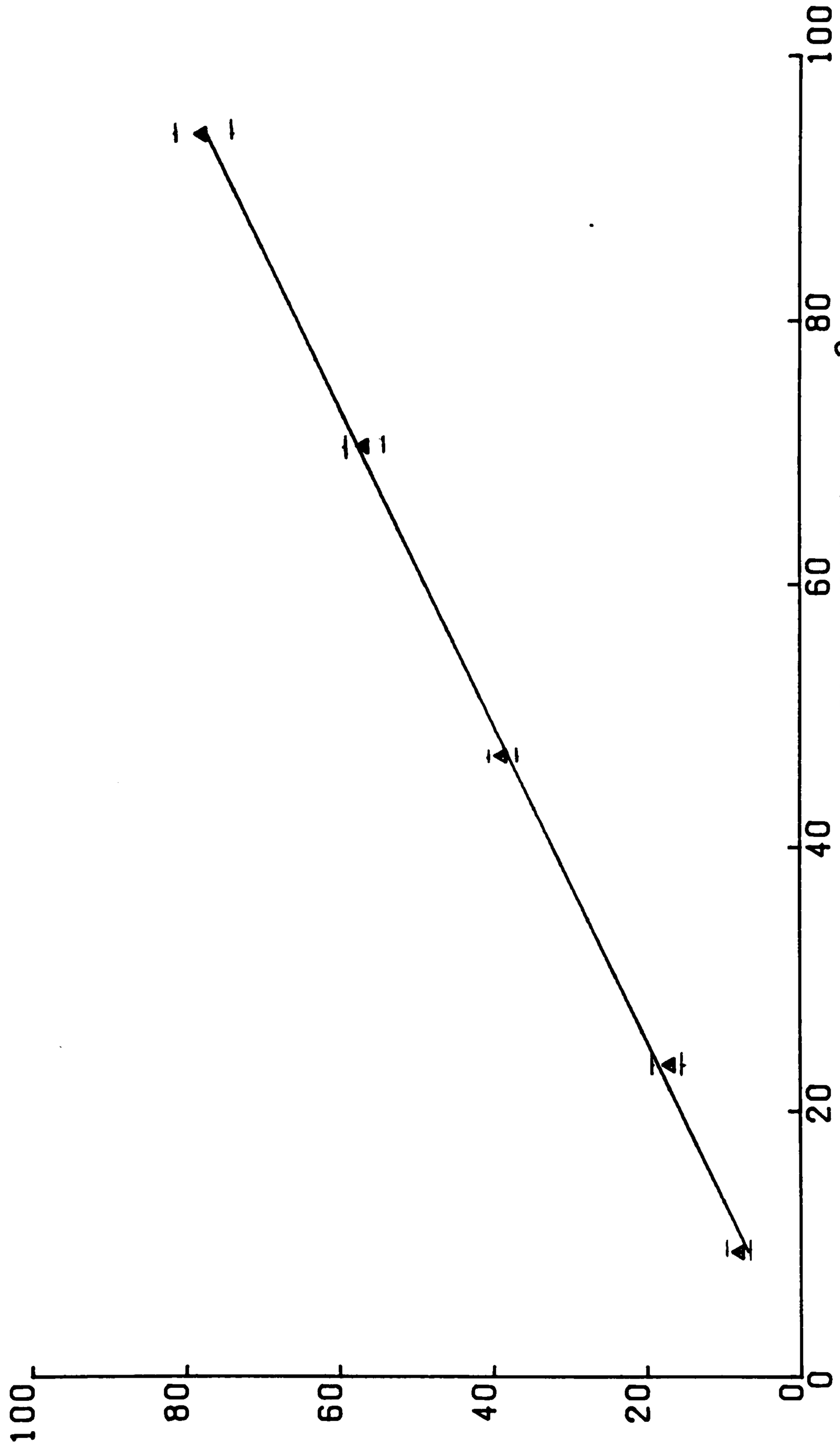
A plot of sulphide concentration vs. peak height is shown in Fig. 13; the 95% confidence intervals are shown on the graph also. A least squares analysis of this data gave the following equation for the straight line:

$$y = 0.83x - 0.77$$

Analysis of Samples

About 15 g of wet sample were weighed into a 250 cm^3 beaker. SAOB (25 cm^3), followed by distilled water (75 cm^3), was

Fig. 13.


CALIBRATION GRAPH FOR G.M.A.S. METHOD
ABSORPTION SIGNAL PK.HT. (mm)
0
20
40
60
80
100
[SULPHIDE](mg dm-3)
60
80
100

added to the beaker and the sample dispersed in the SAOB solution by vigorous stirring. A portion of the resultant suspension was centrifuged, and from a 1 cm^3 aliquot of the supernatant liquid, H_2S was evolved for analysis by injection into 20 cm^3 of hydrochloric acid.

Calculation of Sulphide Concentration in Sample

Five replicate injections were made for each of the samples and the average peak heights of the absorption signals calculated. The average absorption values obtained were substituted into the equation for the straight line of the calibration graph and the concentration of sulphide in the supernatent liquids were thus determined. The concentrations of sulphide in the samples, in units of mg g^{-1} dry weight, were then found from the following equation:

$$\text{vol. supernatent liq. } (dm^3) \times \frac{\text{conc. of sulphide in supernatent (mg } dm^{-3})}{\text{wt. of sample}} \times \frac{100}{\% \text{ dry wt.}}$$

Five portions of sewage sludge, identical with those analysed by Methods 1 and 2, were analysed by the G.M.A.S. method. The results are presented below:-

Sample No.	Sulphide conc. (mg g^{-1})
1	0.80
2	0.61
3	0.74
4	0.65
5	0.80
Average	0.72
Stand. dev.	0.09
Coeff. var.	12.1 %

(4) Potentiometric Titration

Reagents:-

Cadmium nitrate 0.001 mol dm^3 aqueous: Dilute 112.4 cm^3 of a 1000 ppm stock solution (commercially available for A.A. spectrometry) to 1 dm^3.

SAOB: see page 50

Apparatus:-

An Orion Research Inc. Model 94-16 sulphide electrode was used in conjunction with an Orion Model 90-02 double-junction reference electrode. The outer chamber of the reference electrode was filled with 10% KNO_3 solution. A digital voltmeter was used to monitor the potential of the electrode.

Method

Between 0.5 and 1 g of wet sample were weighed into a 250 cm^3 beaker. SAOB (50cm^3) and distilled water (50 cm^3) were added to the beaker and the sample dispersed in the SAOB solution by vigorous stirring. The indicating and reference electrodes were immersed in the solution which was then titrated with cadmium nitrate solution. The solution was stirred continuously throughout the course of the titration by a magnetic follower.

Calculation of Sulphide Concentration in Sample

The change in electrode potential with addition of titrant to form CdS was recorded and a titration curve plotted; the end-point was found from the point of inflection in the curve. A typical titration curve is shown in Fig. 14. Occasionly, some samples produced titration curves with shallow slopes from which it was difficult to locate accurately the point of inflection. In these instances, the end point was located by plotting successive values of the rate of change of cell emf verses each increment of titrant in the vicinity of the inflection point; such a plot is shown in Fig. 15.

Fig. 14

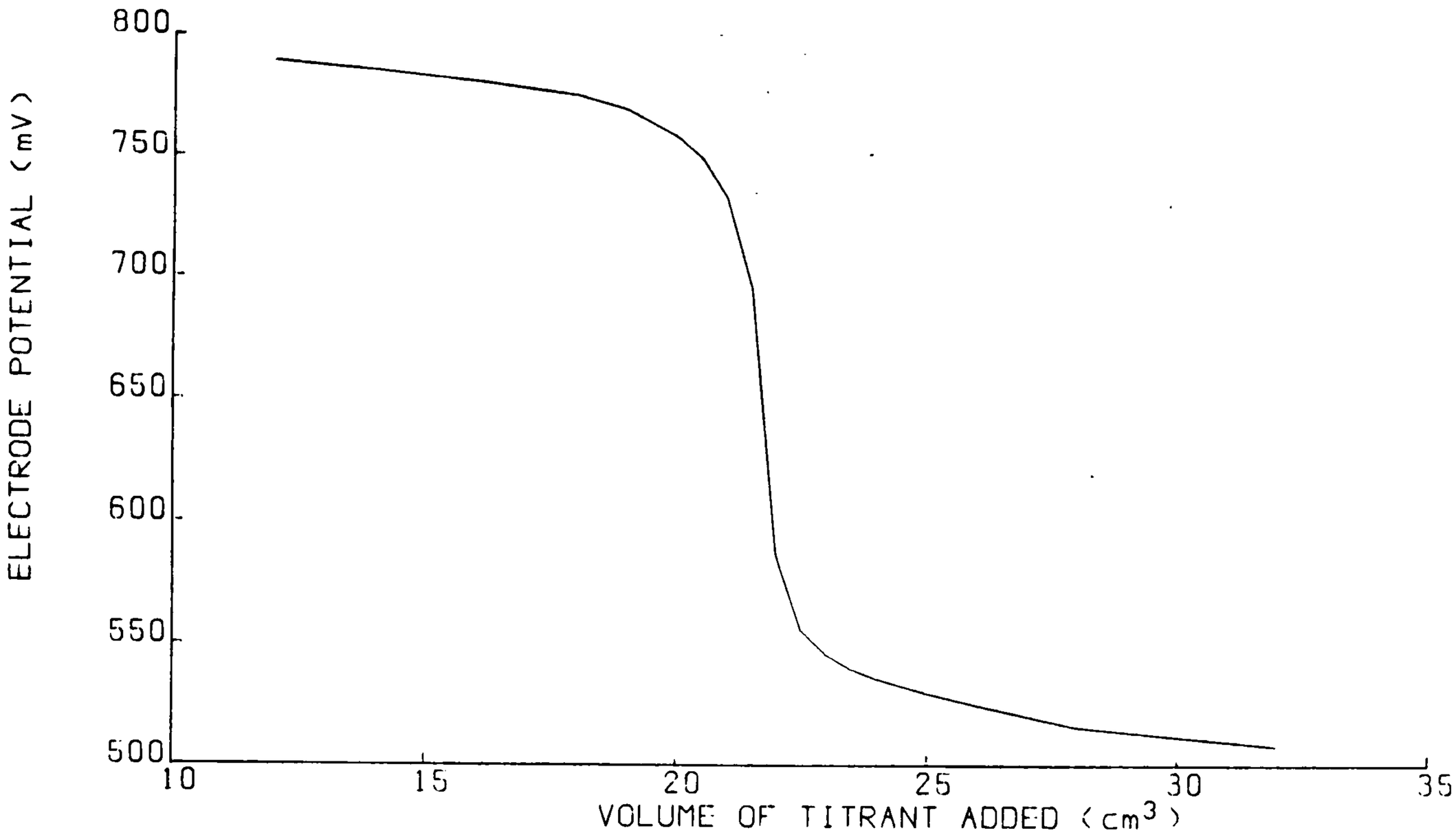
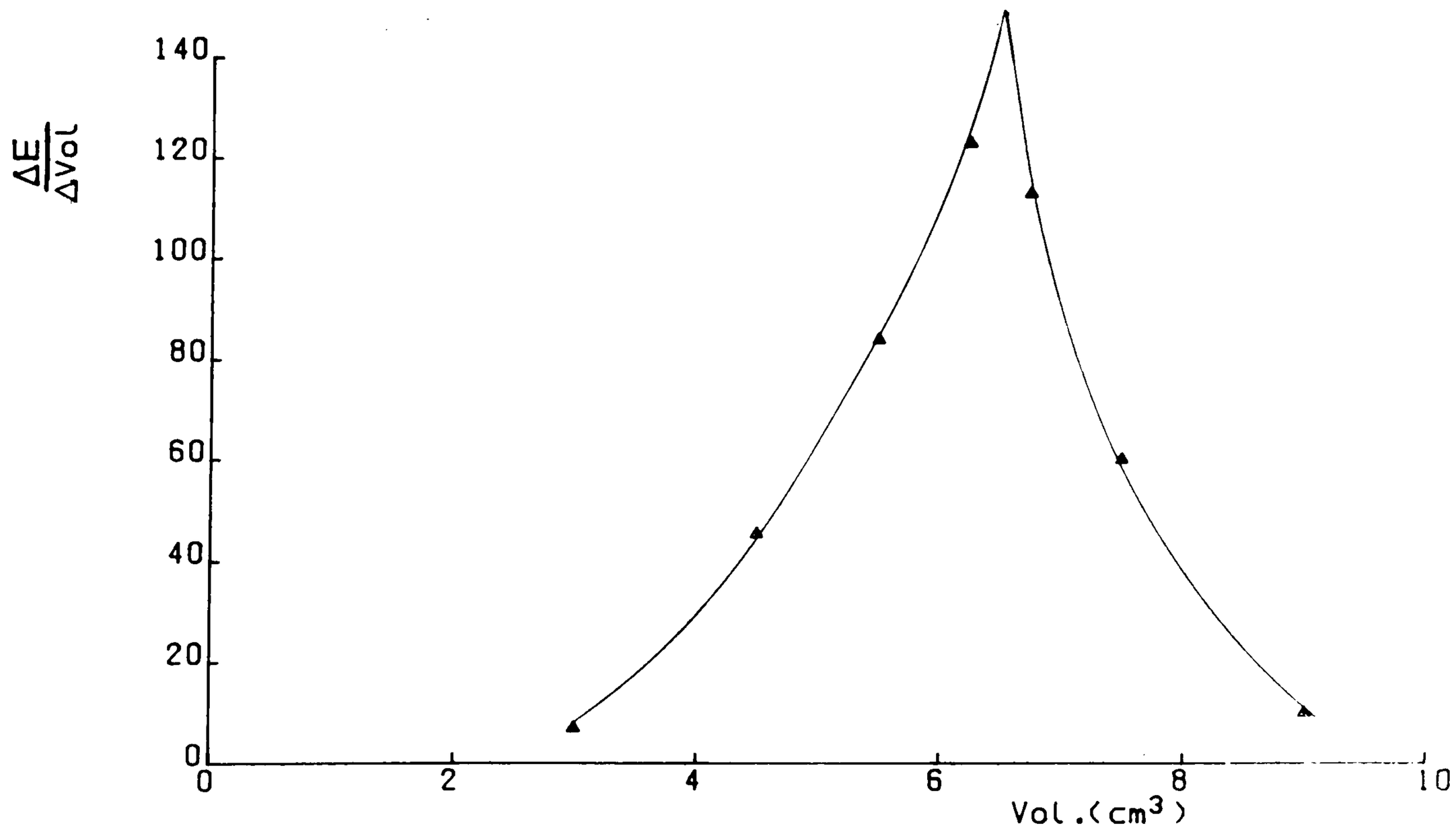


Fig. 15.

FIRST DERIVATIVE TITRATION CURVE



One mole of sulphide ions react with one mole of cadmium ions according to the equation:

$$S^{2-} + Cd^{2+} \longrightarrow CdS$$

The number of moles of sulphide in the sample, therefore, is equal to : titre/1000 x 0.001 moles.

Incorporating the percentage dry weight into the equation and multiplying by 32 000, the concentration of sulphide in the sample, in units of mg g^{-1} dry weight, is equal to:

$$\frac{\text{titre}}{1000} \times 0.001 \times \frac{1}{\text{wt. of sample}} \times \frac{100}{\%\ \text{dry wt.}} \times 32{,}000$$

Five portions of sewage sludge, identical with those analysed by Methods 1,2 and 3, were analysed by the above method. The results are presented below:-

Sample No.	Sulphide conc. (mg g^{-1})
1	0.46
2	0.47
3	0.45
4	0.49
5	0.46
Average	o.46
Stand. dev.	0.01
Coeff. var.	2.5 %

Additional experiments were performed to assess the recovery of added sulphide from sewage sludge; quantitative recoveries (>90%) were obtained for all four methods.

Results and Discussion

The average sulphide concentrations of the homogenised sewage sludge solids obtained by the four methods, together with values for the replicates within the four methods, are presented in Table 5.

Table 5

Wanlip Samples

Method	(Sulphide) (mg g^{-1})	Stand. dev. (mg g^{-1})	Coeff. var.
1. Direct iodometric	1.97	0.10	5.0 %
2. Indirect iodometric	0.83	0.08	9.2 %
3. G.M.A.S.	0.72	0.09	12.1 %
4. Potentiometric titration	0.46	0.01	2.5 %

Method 2 determines both acid-soluble and water-soluble sulphide; Methods 3 and 4 theoretically determine water-soluble sulphide only; whereas Method 1 has been shown to determine water-soluble sulphide and a part of the acid-soluble fraction[103]. On this basis Method 2 would be expected to record the highest sulphide levels. However, the highest levels of sulphide were found from Method 1. Since good sulphide recoveries were obtained from spiked sediments by Method 2 (92 %), it is clear that the direct iodometric method is subject to considerable interference; it has been suggested previously that sulphite and thiosulphate are interferents for this method, and these are present in sediments and sludges[106].

Methods 3 and 4 would be expected to record similar sulphide levels. However, like Cresser[123], the author obtained considerably higher results by the G.M.A.S. method, suggesting that it also is subject to interference. Possible interferents for this method include sulphite, nitrite, sulphide complexes with organic matter and Fe (11), and colloidal iron sulphide which may not have been removed completely in the centrifugation step of the analysis[123,124].

A large variation in the precision of the four methods was found, the most precise results being obtained by Method 4.

This series of experiments was repeated using homogenised

sediments collected from the River Carron (Lothian, Scotland), Cropston Reservoir (Leicestershire) and separate samples of sewage sludge obtained from Wanlip (Leicestershire). The results are presented in Tables 6-8.

Table 6

Cropston Sediments

Method	(Sulphide) ($mg\ g^{-1}$)	Stand. dev. ($mg\ g^{-1}$)	Coeff. var.
1. Direct iodometric	1.73	0.07	4.4 %
2. Indirect iodometric	1.11	0.10	9.3 %
3. G.M.A.S.	0.91	0.08	9.2 %
4. Potentiometric titration	0.83	0.02	2.5 %

Table 7

Carron Sediments

Method	(Sulphide) ($mg\ g^{-1}$)	Stand. dev. ($mg\ g^{-1}$)	Coeff. var.
1. Direct iodometric	2.69	0.14	5.1 %
2. Indirect iodometric	1.43	0.14	9.8 %
3. G.M.A.S.	1.12	0.10	9.1 %
4. Potentiometric titration	1.24	0.03	2.7 %

Table 8

Wanlip Samples (Batch 2)

Method	(Sulphide) ($mg\ g^{-1}$)	Stand. dev. ($mg\ g^{-1}$)	Coeff. var.
1. Direct iodometric	3.63	0.25	6.9 %
2. Indirect iodometric	1.42	0.14	9.8 %
3. G.M.A.S.	1.40	0.14	9.8 %
4. Potentiometric titration	0.93	0.05	4.8 %

The following conclusions can be drawn from the results presented in Tables 5 - 8 :-

1. The lower ratios of results from Methods 2:4 obtained with non-sewage sediments suggests that there is a greater proportion of water-soluble sulphide in non-sewage sediments compared with sewage sludge solids. Ratios of results from Methods 2:4 from Tables 5 - 8 are 1.8, 1.3, 1.2 and 1.5 respectively.

2. Methods 3 and 4 recorded similar levels of sulphide in the analyses of Carron and Cropston sediments, whereas substantially higher levels of sulphide were obtained by Method 3 in the analyses of both batches of sewage sludge. This suggests that the interferents for the G.M.A.S. method are present in higher concentrations in sewage sludge than in sediments. Ratios of results from Methods 3:4 from Tables 1 - 4 are 1.6, 1.1, 1.1, and 1.5 respectively.

3. Method 1 compared to Method 4 also has lower ratios for non-sewage sediments for similar reasons to (1) above. Ratios of results from Methods 1:4 are 4.3, 2.1, 2.2 and 3.9 respectively.

4. The level of precision of a given method remained closely constant over different types of sample, Method 4 being **consistantly** the most precise (average value for the coefficient of variation = 3.1 %)

Method of Sulphide Analysis Adopted in this Project

A method of **determining water-**soluble sulphide in sediments that is accurate, precise and quick was required. Method 2 was rejected as it determines both acid-soluble and water-soluble sulphide, and Method 3 was deemed to be unsuitable as it is imprecise, subject to interferents and slow. Method 1 proved to be the most convenient, but it too was rejected as it is prone to interferents. Method 4 was, therefore, selected as the preferred method: it had the advantages of being precise, relatively immune to interferents and suitable for the analysis of large

quantities of samples.

Finally, as some previous workers investigating methylmercury levels in sediments have reported sulphide levels determined by the direct iodometric method, a comparison between Methods 1 and 4 was undertaken to determine if a general relationship exists between the results obtained by the two methods.

Twenty-two River Carron sediments containing various amounts of sulphide were analysed by Methods 1 and 4; a plot of the data is shown in Fig. 16. A least squares analysis of this data produced the following equation for the straight line : $y = 1.58x + 0.73$, $r = 0.92$ ($p<0.001$). Similar comparisons were made on sediments obtained from the River Clyde, River Teign and River Dart; the equations for the straight lines and the linear correlation coefficients obtained are presented below.

Clyde sediments $y = 2.06x + 0.01$, $r = 0.95$ ($p<0.001$)

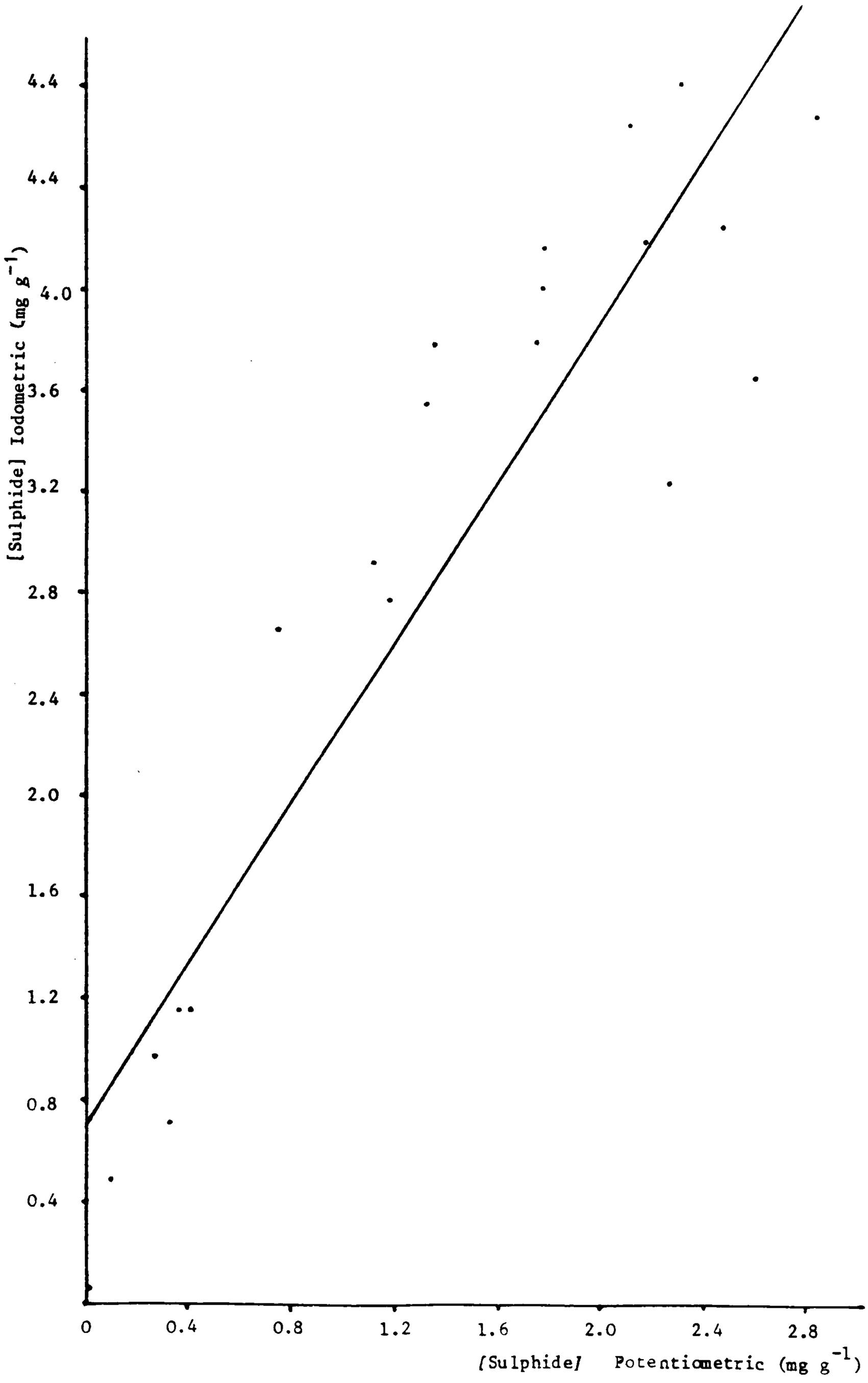
Teign " " $y = 2.47x + 0.30$, $r = 0.95$ ($p<0.001$)

Dart " " $y = 1.88x + 0.37$, $r = 0.90$ ($p<0.001$)

It has, therefore, been shown that an approximately linear relationship exists between the results obtained by the two methods, and that for sediments of a given river, results obtained by Method 1 may be extrapolated statistically to produce approximately true sulphide levels.

Fig. 16.

[Sulphide] Direct Iodometric vs. *[Sulphide]* Potentiometric



Chapter 7

The Determination of Total Mercury in Sediment Samples

The method employed during the course of this project for the determination of total mercury in sediment samples was based on procedures developed by Norton and Bartlett. The method involved (a) a sample pretreatment step, in which the sediment was digested in a mixture of acids and oxidising agents, followed by (b) the determination of mercury in the digest using the technique of cold-vapour atomic absorption spectrometry (C.V.A.A.).

(a) Digestion Procedure

During the course of the digestion procedure, various forms of mercury present in the sample were converted to Hg (11). The procedure is discussed below after the method used to clean the glassware employed in the digestion step is first considered.

Cleaning of Glassware

Because of the ease at which mercury can become adsorbed to glass, it was necessary to ensure that all glassware used in the analytical procedure was scrupulously clean. This was achieved by soaking the glassware in aqua regia (3 parts S.L.R. grade conc. hydrochloric acid to 1 part S.L.R. grade conc. nitric acid). Beakers used for sediment digestion were soaked for 4 hours in aqua regia and then washed with tap water, followed by distilled water, and finally dried at 200°C. Volumetric flasks and centrifuge tubes were treated similarly but were left wet after rinsing with distilled water.

Reagents

Aqua regia: Mix 3 volumes of 'Aristar' conc. hydrochloric

acid with 1 volume of 'Aristar' conc. nitric acid. Prepare this reagent immediately before use.
Potassium dichromate (A.R.) 10 % w/v aqueous.
Potassium persulphate (A.R.) 5 % w/v aqueous.

Digestion Method

Between 2 and 5 g of wet sediment were weighed into a 250 cm^3 beaker. A 25 cm^3 portion of freshly prepared aqua regia was added to the beaker and, after the initial reaction had subsided, the mixture was boiled for 1 minute in a fume cupboard. The beaker was then removed from the hot plate and 20 cm^3 of potassium dichromate solution was added to it. The solution was boiled again for 3 minutes and then allowed to cool when a further 5 cm^3 of potassium dichromate solution and 2 cm^3 of potassium persulphate solution were added to the beaker.

At this stage Morton[102] and Bartlett[103] transferred the complete digest, including solids, to a 100 cm^3 volumetric flask. This resulted in the incurrence of a volume error of about 5 % which had to be taken into account in the final calculation. Such a procedure had been employed as Morton[102] had found that filtration of the sediment digest before dilution to 100 cm^3 led to loss of mercury to the filter. At the beginning of this project the same procedure was used, but later a centrifugation step was incorporated into the method to remove solid material. The digestion mixture was centrifuged at 5000 rpm for 3 minutes, the supernatent liquid was then transferred to the volumetric flask, the centrifuge tubes were washed with several portions of distilled water and the washings added to the flask before the volume was made up to the mark. Using this procedure it was found that solids could be removed from the digestion mixture without incurring loss of mercury.

A reagent blank was run in parallel with the above procedure.

(b) Cold-vapour Atomic Absorption Spectrometry

In this part of the analysis, an aliquot of the digestion solution was pipetted into a reduction vessel, where Hg (11) present in the solution was reduced to Hg^{o} by the addition of stannous chloride. Elemental mercury was then swept out of the vessel by a stream of nitrogen and led into a flow-through cell situated in the light path of a mercury hollow cathode lamp. The absorbance of the mercury atoms at 253.7 nm was measured and recorded as a peak on a chart recorder; the amount of mercury present in the original sample was then found from the peak height (see calculation below). The C.V.A.A. apparatus is illustrated in Fig. 17.

Apparatus and Instrumentation

An Instrumentation Laboratory model 151 atomic absorption spectrometer modified for cold-vapour analysis was used throughout the project.

A Quickfit test-tube, capacity 50 cm^3, was used as the reduction vessel.

The flow-through cell was made of glass, 12 cm long and 1.5 cm internal diameter, with quartz windows fitted to both ends.

The carrier gas flow rate was controlled by a GEC-Marconi Rotameter.

The cell was connected to the reduction vessel with a short piece of polythene tube to minimise dead volume.

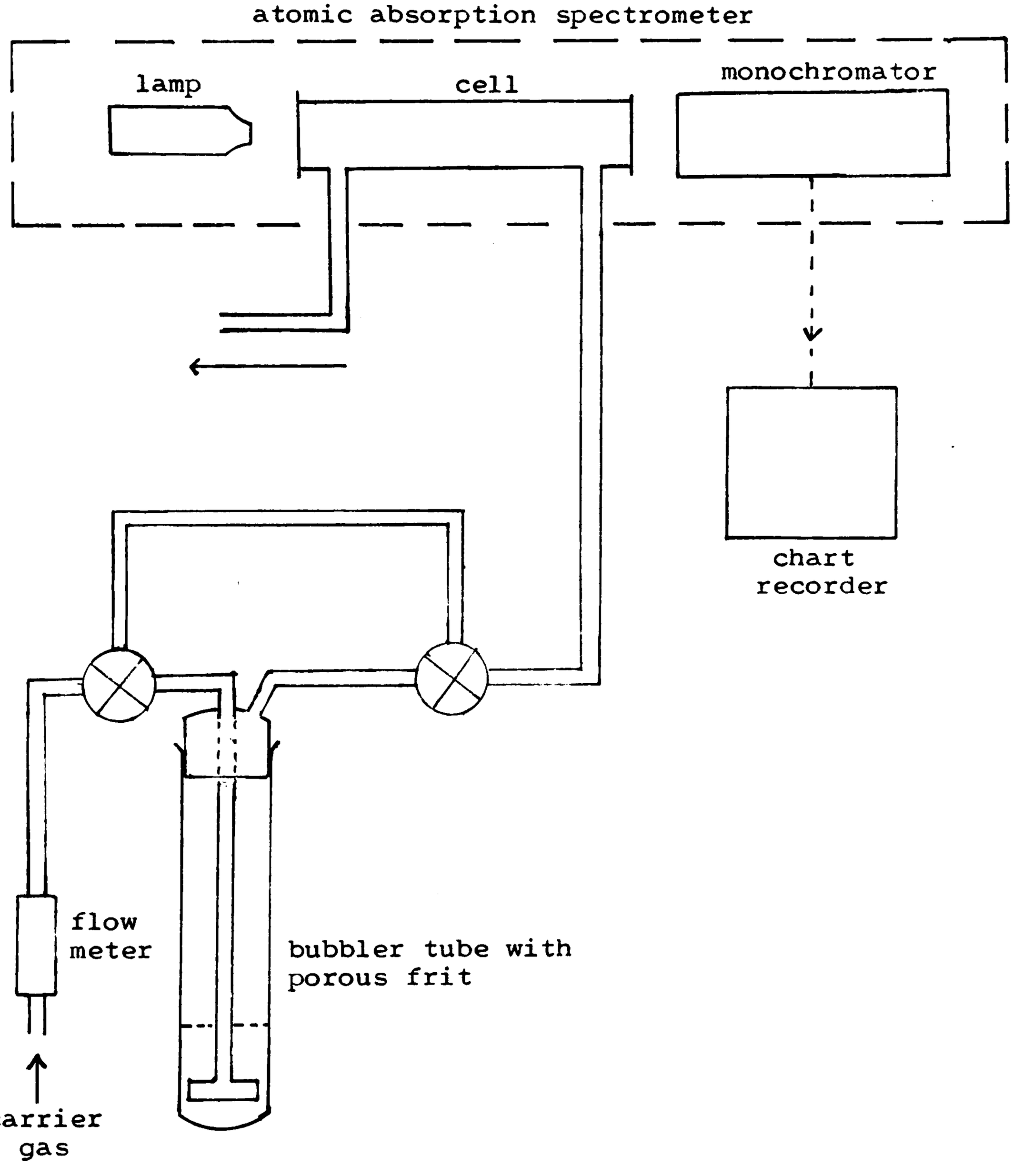
Reagents

Reduction solution: 20 % w/v stannous chloride (A.R.) suspended in 5 % w/v sulphuric acid (A.R.).

Standard mercury solutions: Special precautions need to be taken when preparing standard mercury solutions as mercury can be lost readily through volatilisation, absorption into vessel walls and suspended colloidal material,

Fig. 17.

Cold-vapour Apparatus for the Determination of Total Mercury



or by incorporation into stable complexes[134,135]. It is, therefore, necessary to include preserving agents when preparing standard mercury solutions. In this project, the recommendations of Feldman[136] were adopted; mercury solutions were prepared in distilled water containing 5 % v/v nitric acid (A.R.) and 0.01 % w/v potassium dichromate (A.R.) and stored in glass vessels. Working standards containing 10 - 100 ug dm^{-3} of mercury were prepared by the following procedure:-

(1) 10 cm^3 of a mercury stock solution of concentration 1000 ppm (commercially available from BDH Chemicals Ltd.) were diluted to 100 cm^3 with acid/dichromate solution (soln. 1).

(2) 1 cm^3 of soln. 1 was further diluted to 100 cm^3 with acid/dichromate solution (soln.2).

(3) 1, 2.5, 5, 7.5 and 10 cm^3 portions of soln. 2 were diluted to 100 cm^3 with acid/dichromate solution to produce mercury standards of concentration 10, 25, 50, 75 and 100 ug dm^{-3} respectively. Pipettes and volumetric flasks were used in the preparation of all solutions.

Method

An 8 cm^3 aliquot of distilled water was pipetted into the reduction vessel together with 1 cm^3 of the reduction solution. The vessel was connected to the apparatus, as illustrated in Fig. 17, and a flow of carrier gas bubbled through the solution. Normally a small peak appeared on the recorder at this time, due to traces of mercury present either in the stannous chloride solution or the reduction vessel from the previous analysis. When the pen had returned to the baseline, the carrier gas was directed round the bypass, the reduction vessel was disconnected and a 1 cm^3 aliquot of a standard or sample solution pipetted into it. The reduction vessel was re-attached to the apparatus and the reduced mercury swept through into the

absorption cell. After the peak maximum had been recorded, the carrier gas was diverted round the bypass to allow the pen to return to the baseline quickly. The reduction vessel was disconnected, rinsed several times with water and then used for the next analysis.

The following operating conditions were employed throughout the period of study:-

Monochromator setting	:	253.7 nm
Bandwidth	:	1 nm
Carrier gas flow rate	:	700 cm^3 min^{-1}

The lamp current was set to ~ 20 mA but the exact setting could not be guaranteed. The photomultiplier gain and chart recorder sensitivity were adjusted so that a 100 ng Hg standard produced a full scale deflection.

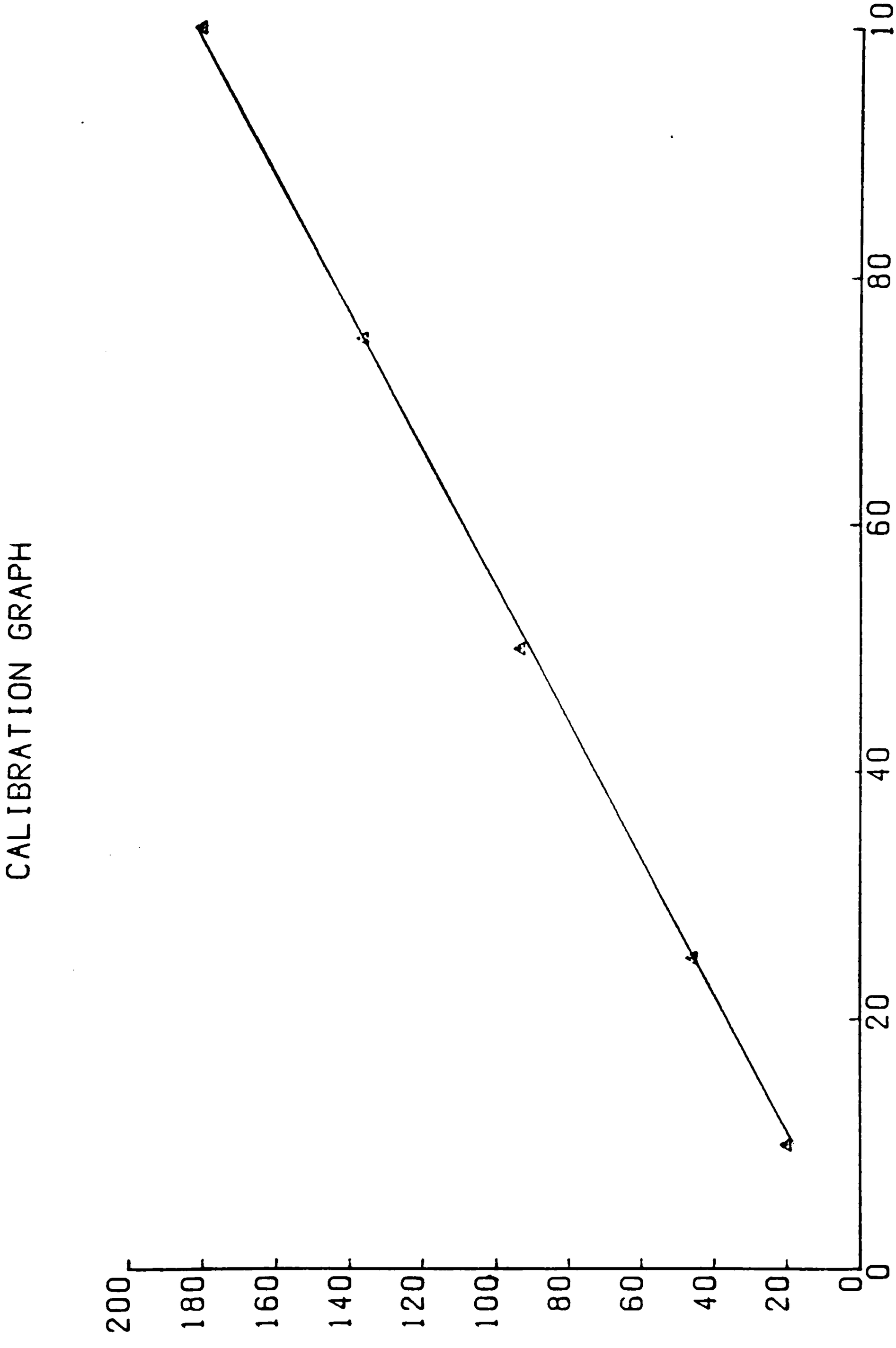
Calibration of the System

A calibration curve (Fig. 18) was prepared by using $1 cm^3$ aliquots of each of the working Hg standards in the procedure detailed above and plotting peak height of the absorption signal against mercury concentration. Each of the points shown in Fig. 18 is the average of 5 readings and the best line was drawn through the points using the method of least squares. It can be seen from the calibration graph that the relationship between mercury concentration and peak height is linear up to a concentration of at least 100 ng Hg.

Calculation of Results

The amount of mercury in the sample solutions was determined by refering the measured values of absorbance to the standard working curve. The procedure used was recommended by ASTM[137] and had the advantage of being able to detect instrumental drift. The procedure is described below.

Fig. 18.


CALIBRATION GRAPH
ABSORBTION SIGNAL PEAK HEIGHT (mm)
0
20
40
60
80
100
120
140
160
180
200
[Hg] (ng)
40
60
80
100

The standard deviation of six measurements using the 100 ug dm^{-3} Hg standard was determined. The absorbance of each of the calibration solutions was then measured. Finally, the absorbance of the sample solutions was measured in batches of 4. After each group of 4 absorbance measurements had been made, the absorbance of the 100 ug dm^{-3} Hg standard was again determined and if the value differed from the average of the 6 values by more than twice the standard deviation, the cause of the drift was determined and the procedure repeated beginning with the calibration. Only once during the course of this work was it necessary to repeat the procedure, the probable cause of the drift being insufficient time allowed for the electronics of the instrument to warm up.

The amount of mercury present in 1 cm^3 aliquots (x) of the sample solutions was read off from the working curve. A value for the concentration of mercury in the original sediment samples was then found from the following equation:

$$x(ug) \times 100 \times \frac{1}{\text{wt. of sample}} \times \frac{100}{\% \text{ dry wt.}} \quad (ug\ g^{-1} \text{ dry wt.})$$

Precision of the Method

The precision of the method was determined by making 5 replicate analyses on a single sediment sample. The results are presented below together with values for the standard deviation and coefficient of variation.

Sample No.	Mercury Conc. (ug g^{-1})
1	2.96
2	2.80
3	3.10
4	2.95
5	2.73
Average	2.91
Stand. dev.	0.15
Coeff. var.	5.00 %

Recovery of Mercury

Experiments were performed to assess the recovery of mercury from spiked samples. These experiments were performed using 2 g portions of a dried, homogenous sediment which was known - from 10 replicate analyses - to contain 0.53 ug g^{-1} of Hg. Five portions of sediment were spiked with 2 ug of mercury (1 cm^3 of a solution containing 2 mg dm^{-3} mercury in the form of mercuric nitrate), and 5 portions of sediment were spiked with 8 ug of mercury (4 cm^3 of the mercuric nitrate solution). The sediments were analysed for mercury content by the method described; the average results obtained for the two sets of 5 replicates are presented below.

Mercury Present	Mercury Added	Mercury Found	% Recovery
1.06	2 ug	2.97	97.1
1.06	8 ug	8.73	96.4

The percentage recoveries were close enough to 100 % to be deemed satisfactory.

Limit of Detection

The limit of detection of the method was ascertained by determining the concentration corresponding to twice the standard deviation of 10 blank absorbance measurements. The results are presented below.

Average peak height = 7.1 mm

Standard deviation = 0.9245 mm

Blank concentration = 4.1 ng cm^{-3} Hg

The limit of detection was thus:-

$$\frac{2 \times 0.9245}{7.1} \times 4.1 = 1.07 \text{ ng cm}^{-3}$$

Assuming that the amount of dry sediment used in a typical

analysis was 2 g, the limit of detection can be expressed as 0.05 ug g^{-1}.

Sensitivity

The sensitivity of the method is defined as the concentration of solution that produces an absorbance of 0.004 measured near the origin. Five absorbance measurements made using a 10 ug dm^{-3} Hg standard gave a mean value of 0.02157. The sensitivity of the method was thus:

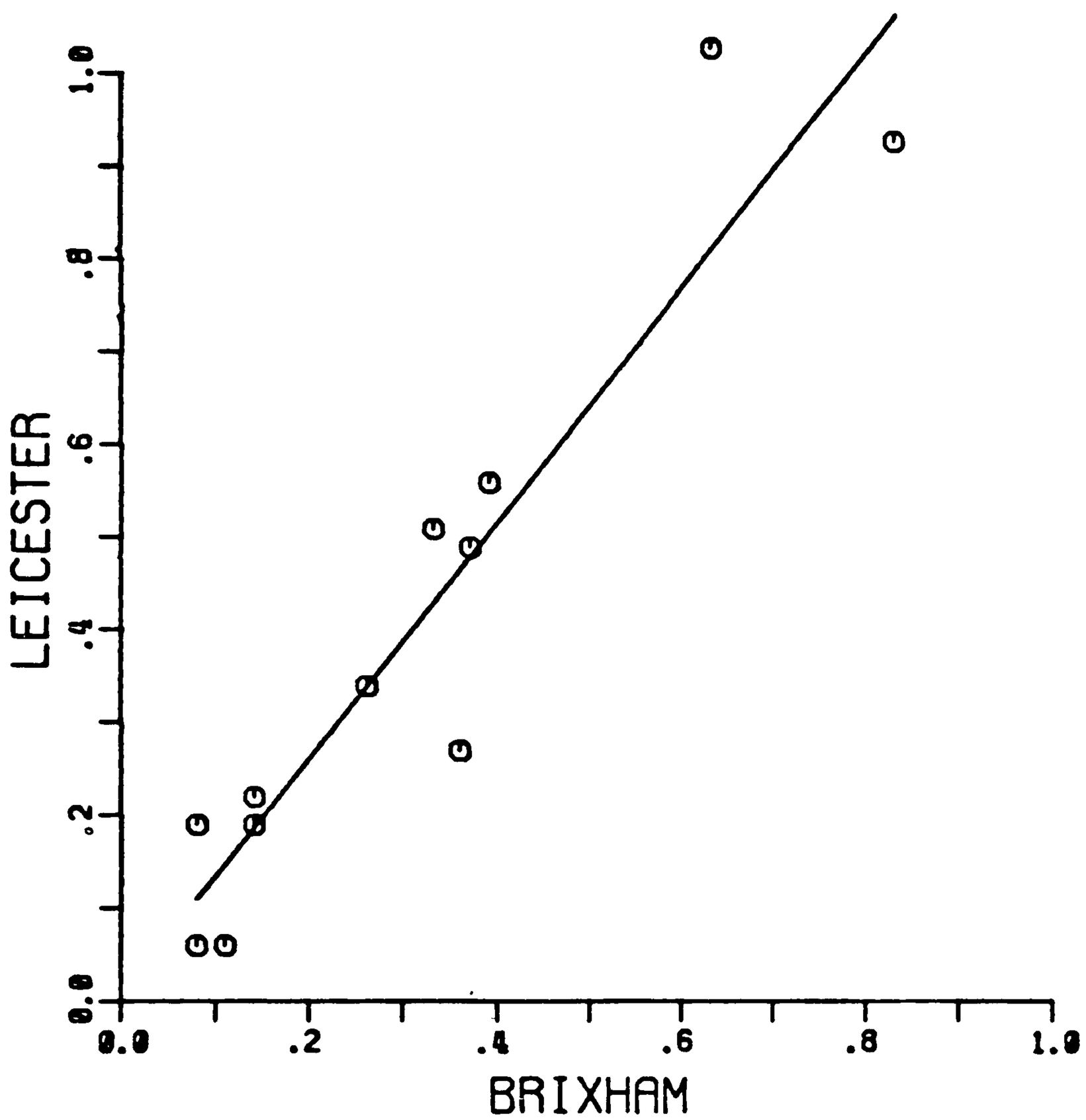
$$\frac{0.0044}{0.02157} \times 10 = 2 \text{ ng cm}^{-3}$$

Inter-laboratory Correlation

During the course of the project, 12 sediment samples, taken from estuaries in South West England, were analysed for total mercury by the method described and by the method employed at the I.C.I. Brixham Laboratory. The graph of this is shown in Fig. 19. A least squares analysis of the data produced values of 0.94 ($p<0.001$) for the correlation coefficient and 1.28 for the slope of the line. The close correlation was very encouraging. The Brixham method of analysis employed an aqua regia digest followed by C.V.A.A. The slightly higher values obtained by the method described - as shown by the value of the slope - may have been due to the presence of oxidising agents in the digestion mixture, preventing reduction of Hg (11) to Hg^{O} and subsequent loss of mercury through volatilisation.

Fig. 19.

$[Hg]_{TOT.}$ Intercalibration (ug g^{-1})



Chapter 8

The Determination of Methylmercury in Sediments

Methylmercury levels in sediments were determined by a method which was derived from procedures developed by Morton[102] and Bartlett[103]. The method involved the extraction of methylmercury from sediments through a number of clean-up steps, before final separation and determination of methylmercury on a gas chromatograph fitted with an electron capture detector.

The method is discussed in two parts : (a) clean-up procedure, (b) gas chromatography.

(a) Clean-up Procedure

The clean-up procedure used involved 3 extraction steps. Methylmercury in the sediment was first converted into methylmercuric bromide by the addition of bromide reagent (see below); copper ions were also added to the reaction mixture at this stage to mask sulphur groups in the sediment and thus facilitate the release of methylmercury. The methylmercuric bromide formed was extracted into toluene and then back extracted into aqueous thiosulphate solution. The aqueous solution was then treated with strong copper bromide reagent, following which the methylmercury was extracted as the bromide into 'Aristar' toluene for determination by electron capture gas chromatography.

This clean-up procedure was based on the method developed by Morton[102], and differed from Morton's procedure in that 'Aristar' toluene, rather than 'Ultrar' benzene, was used in the final extraction step for safety reasons. At the outset of this project it was decided to ascertain if Morton's method could be modified by using Aristar toluene in place of Ultrar benzene; a previously published paper

by the Analytical Methods Committee[138] suggested that both solvents may be used for the extraction of methylmercury.

Experiments showed that 'Aristar' toluene contained no impurities which would produce interfering peaks on the chromatogram. Experiments were then undertaken to assess the extraction efficiencies of both solvents for methylmercury. The extraction procedure used is detailed below:

Reagents :-

Toluene (A.R.)
Benzene (Ultrar)
Toluene (Aristar)
Copper sulphate (A.R.) 25 % w/v aqueous
Sodium thiosulphate (A.R.) 0.005 mol dm^{-3} aqueous
Bromide reagent: mix 110 cm^3 of conc. sulphuric acid (A.R.) with 100 cm^3 of distilled water and allow to cool. Dissolve 360 g of potassium bromide (A.R.) in 700 cm^3 distilled water and make up volume to 1 dm^3.

Method

Between 2-5 g of wet sediment were weighed out into a 35 cm^3 centrifuge tube and the sample volume made up to ~10 cm^3 with distilled water. Exactly 8 cm^3 of toluene (A.R.) was added to the tube followed by 1 cm^3 of copper sulphate solution and 4 cm^3 of bromide reagent. After the initial effervescence had subsided, the tube was stoppered and shaken vigorously for 2 minutes, then centrifuged to separate the solid, aqueous and organic phases. Exactly 4 cm^3 of toluene was withdrawn and transfered to a 10 cm^3 centrifuge tube, and this was extracted twice with 3 cm^3 portions of the thiosulphate reagent. The aqueous extracts were removed by a Pasteur pipette and combined in another centrifuge tube. The thiosulphate extracts were treated with 0.2 cm^3 of copper sulphate solution, 1 cm^3 of bromide reagent and exactly 1 cm^3 of 'Ultrar' benzene or

'Aristar' toluene. The mixture was shaken vigorously for 2 minutes and the organic layer then removed and stored in a 5 cm^3 volumetric flask before analysis.

The clean-up procedure detailed above was followed through for 2 identical sets of sediment samples. Each set consisted of 5 portions of sediment, identical in weight, but containing various amounts of added methylmercury. 'Aristar' toluene was used in the clean-up procedure of one set of samples, and 'Ultrar' benzene was used in the clean-up procedure of the other set. The final extracts were analysed by electron capture gas chromatography.

The results for this experiment are summarised in Figs. 20 and 21. Straight lines were drawn through the points in the graphs following a least squares analysis of the data, and the following values were obtained for the gradients of the lines:-

Toluene : gradient = 0.925, i.e. 92.5 % recovery

Benzene : gradient = 0.965, i.e. 96.5 % recovery

This experiment was repeated several times, and in all cases slightly higher recoveries of methylmercury were obtained using benzene. The difference in the results may be due, at least in part, to the greater volatility of benzene, as the higher rate of evaporation of this solvent during the work-up procedure would lead to a greater "concentration effect" being incurred. However, as only slightly poorer recoveries of methylmercury were obtained with toluene, it was decided that the use of this solvent in future work was justifiable because of its lower toxicity.

(b) Gas-chromatography

A Pye 104 chromatograph fitted with a ^{63}Ni electron capture detector was used throughout this project. The instrument was fitted with a glass column, 4 mm diameter, with a Pye glass to metal seal at the exit. The column

Fig. 20.

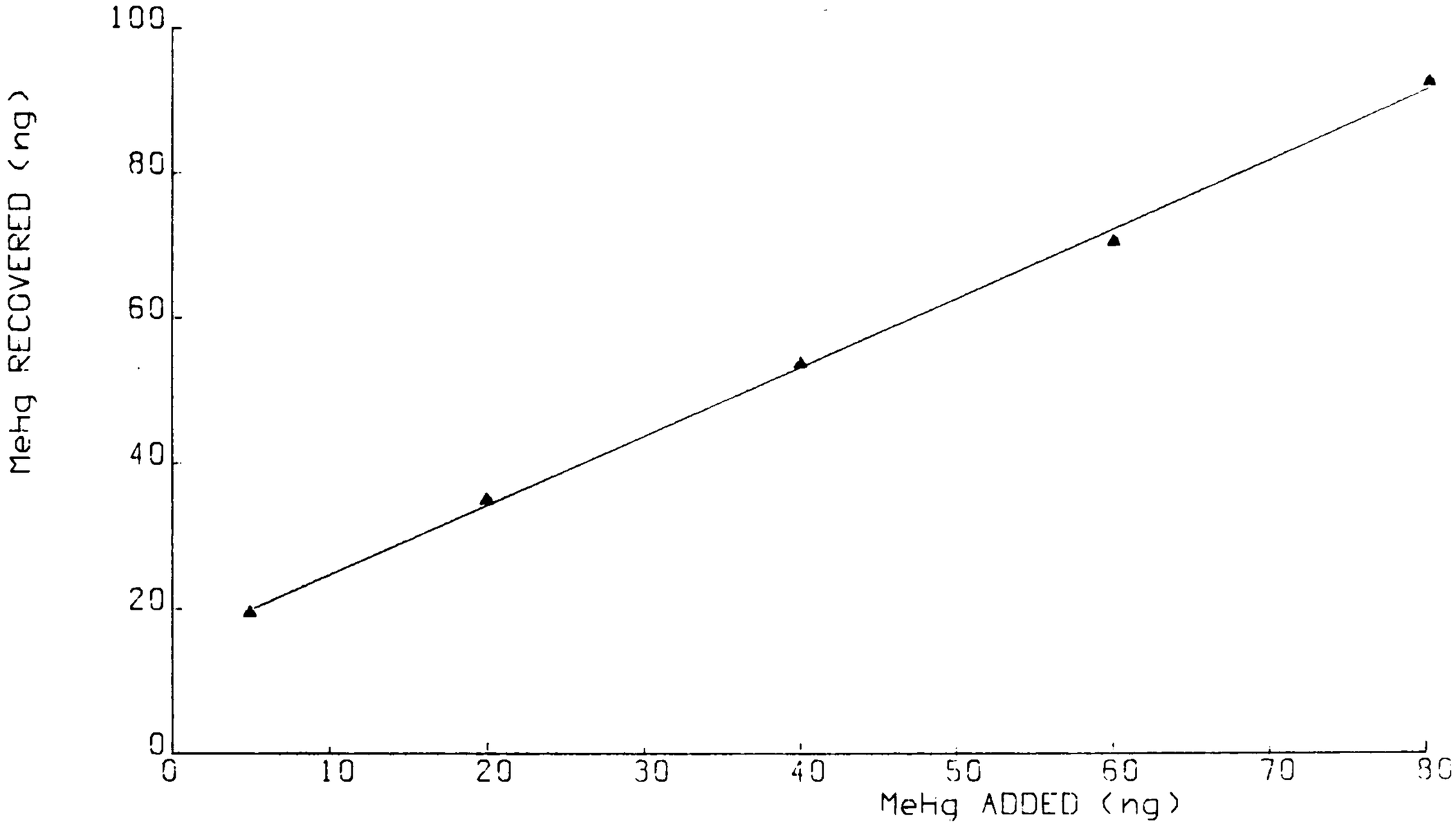
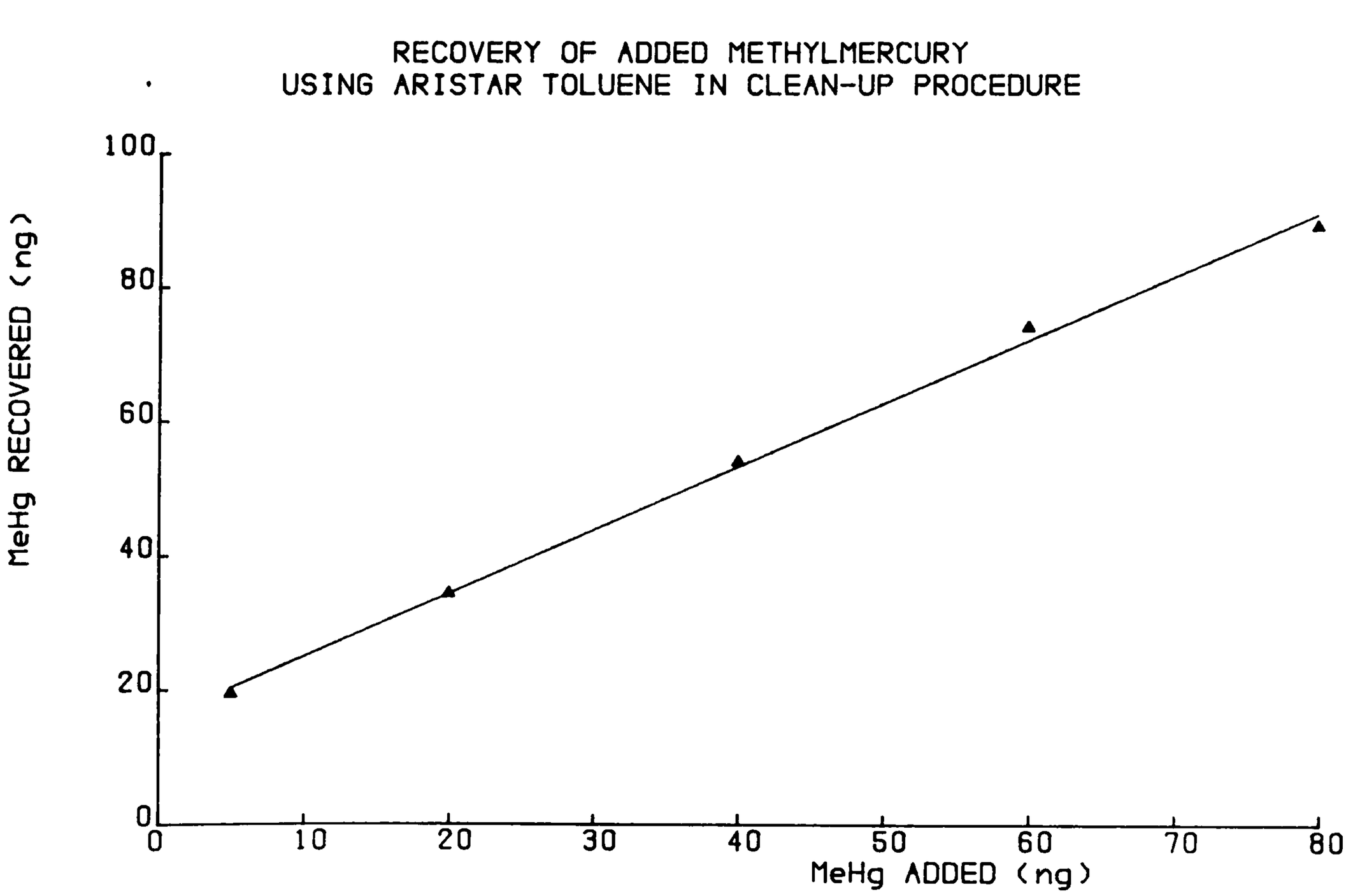

RECOVERY OF ADDED METHYL MERCURY,
USING ARISTAR BENZENE IN CLEAN-UP PROCEDURE
MeHg RECOVERED (ng)
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
MeHg ADDED (ng)

Fig. 21.


RECOVERY OF ADDED METHYLMERCURY
USING ARISTAR TOLUENE IN CLEAN-UP PROCEDURE
MeHg RECOVERED (ng)
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
MeHg ADDED (ng)

was packed with 5 % monoethylene glycol adipate on Chromosorb G (A.W.) 80 - 100 mesh (available commercially from Phase Separations Ltd.). The length of the column varied throughout the course of the project (see below). 'White Spot' oxygen free nitrogen was used as the carrier gas, and a molecular sieve was incorporated into the gas line to provide a further purity safeguard. The detector was always used in the pulse mode, usually with a pulse time of 500 μs.

In the early part of this project, the operating conditions used by Bartlett[103] were employed; these are detailed below:-

Column length	:	1.0 m
Injection temp	:	110°C
Column temp.	:	165°C
Detector temp.	:	265°C
Flow rate	:	100 cm^3 min^{-1}

subsequently, it was found that better results could be obtained by injecting directly on to the column, and by using a longer column, a higher column temperature and a faster carrier gas flow rate. The operating conditions used in the final part of the project are detailed below:-

Column length	:	1.6 m
Column temp.	:	175°C
Detector temp.	:	265°C
Flow rate	:	120 cm^3 min^{-1}

A typical chromatogram obtained using these conditions is illustrated in Fig. 22. From Fig. 22 the adjusted retention time for methylmercury - i.e. the time between the elution of an unretained sample (i.e. the solvent front) and methylmercury - is found to be 4.2 mins. Resolution (R_s) can be calculated from the standard formula :

Fig. 22.

Typical Chromatogram Obtained from 1 u dm^3 Injection of Final Toluene Extract

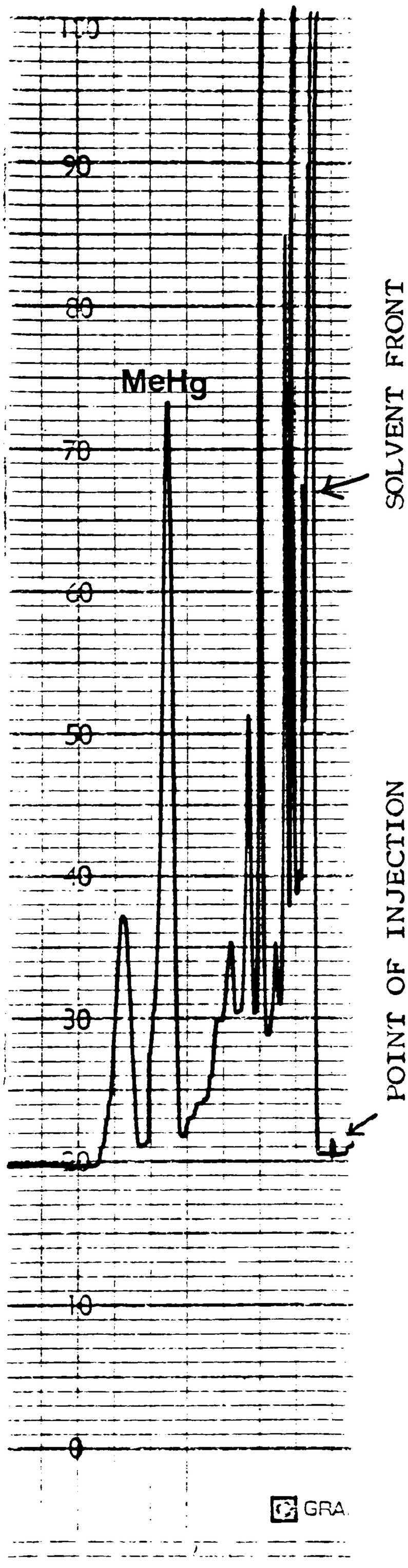


Chart Speed = 5 mm min^{-1}

$$Rs = 2\left(\frac{tR_2 - tR_1}{w_2 + w_1}\right) \qquad (139)$$

where tR_1 is the retention time of methylmercury, tR_2 is the retention time of the closest peak, w_1 is the width of the methylmercury peak and w_2 is the width of the nearest peak. From Fig. 22, a value of 1.5 is calculated for Rs, indicating complete baseline resolution.

The efficiency of the column (N) is calculated from the following equation :

$$N = 5.54\left(\frac{tR}{w_{\frac{1}{2}}}\right)^2 \text{ theoretical plates,}$$

where $w_{\frac{1}{2}}$ is the width of the methylmercury peak at half the peak height. From Fig. 22, a value of 611 theoretical plates is calculated for N.

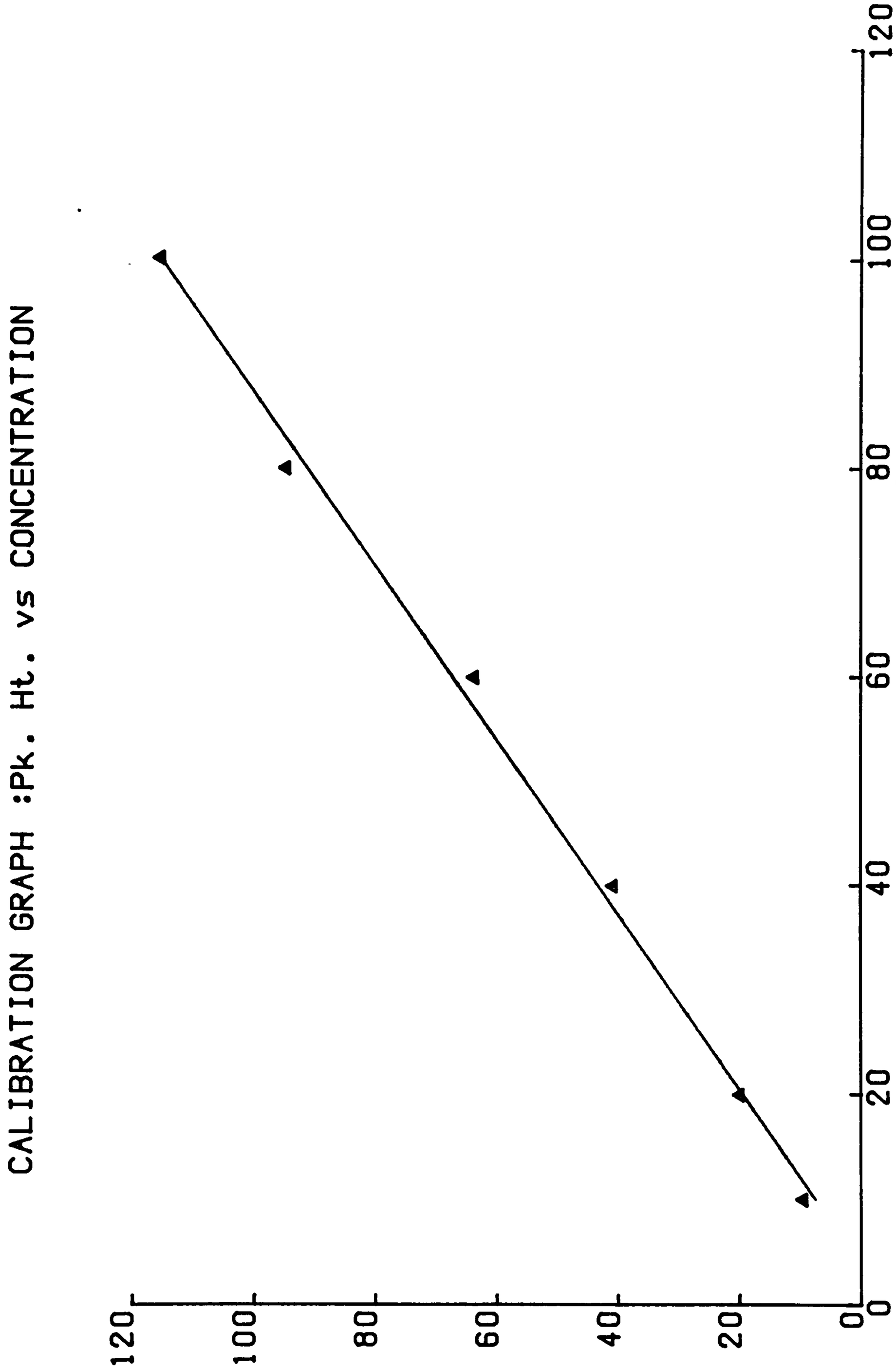
It was found that the performance of the column deteriorated after about 700 hours use, and complete replacement with a fresh column was then necessary. New columns were conditioned before use by running at 190°C for 4-5 days, with a flow rate of 60 cm^3 min^{-1} of nitrogen.

Calibration of the Chromatographic Method and Calculation of Results

A calibration graph was prepared by injecting on to the chromatograph 1 ul aliquots of methylmercury standards covering the range 10-100 ng dm^{-3}, and then plotting methylmercury peak heights against concentration. The calibration graph is shown in Fig. 23. From Fig. 23 it can be seen that the analytical growth curve is linear up to 100 pg of methylmercury at least.

For environmental samples the extraction procedure described on pp 68-69 was followed through, and a 1µl aliquot of the final toluene extract was injected on to the

Fig. 23


CALIBRATION GRAPH :Pk. Ht. vs CONCENTRATION
Pk. Ht. (mm)
[MeHg](pg)
0
20
40
60
80
100
120

chromatograph. The height of the methylmercury peak obtained from the sample was compared to that obtained from a 1 µl injection of a methylmercury standard. If the peak height of the sample was greater than that of a 100 pg standard, then the final extract was diluted further with solvent until a peak height below that of the 100 pg standard was obtained (this was not necessary for natural environmental samples). A reagent blank was run in parallel with the above procedure.

The concentration of methylmercury in the sample is therefore given by the following formula following from the operations:

$$\frac{\text{Pk. ht. sample} - \text{Pk. ht. blank}}{\text{Pk. ht. std.}} \times \text{conc. std.} \times 2 \times \frac{1}{\text{wt. of sample}} \times \frac{100}{\% \text{ dry wt.}} \quad \text{ng g}^{-1}$$

Precision of the Method

Five identical portions of sediment were analysed by the above procedure. The results are presented below together with values for the standard deviation and coefficient of variation.

Sample No.	Methylmercury (ng g^{-1})
1	28.7
2	24.3
3	27.6
4	25.1
5	24.8
Average =	26.1
Std. dev. =	1.9
Coeff. var. =	7.4 %

Limit of Detection

The instrument was just capable of detecting methylmercury in a 10 µl injection of a 0.2 ug dm^{-3} standard (this produced a signal to noise ratio of 2). However, in practice the limit of detection was governed by the presence of impurities in the extracts. Usually, it was possible to detect 1 ng of methylmercury in the final 1 cm^3 toluene extract. Assuming that 5 g of dry sediment was the maximum that could be extracted by the above procedure, the limit of detection of the method is 0.4 ng g^{-1}.

Chapter 9

Redox Potential Measurements

Introduction

The redox potential of a chemical system provides a measure of the ratio of oxidised to reduced species in the system. Such potentials can be measured by an inert metal electrode (most commonly platinum) used in conjunction with a reference electrode to form a complete cell. The redox potential (Eh) is obtained from the potential of the cell (E) by adding the appropriate value for the reference electrode potential corrected for liquid junction effects.

The measurement of redox potentials of sediments using such electrodes is, however, subject to a number of problems. Whitfield[119] has reviewed these problems in some detail. Five principal problems can be identified and these are discussed briefly below:-

(1) The insertion of electrodes into a sediment can disturb the environment by either releasing gases or introducing air.

(2) The liquid junction between the solution in the reference half-cell and the sample solution can give rise to spurious potentials, due to either (a) the effects of flocculent suspended material or (b) the decomposition of heavy metal sulphides at the liquid junction following reactions between hydrosulphide ions in the sediment and cations of the sparingly soluble salt used in the internal reference electrode.

(3) Natural systems contain many redox couples that are not at equilibrium, and are unable to attain equilibrium at the electrode surface and control the electrode potential. In fact, some components such as sulphide may

attack the metal surface and give rise to irreversible reaction potentials.

(4) Since redox potentials depend upon the ratio of oxidised to reduced forms in the system, and not on their absolute concentration, a rapidly reversible redox reaction that has little significance in the overall chemistry of the environment may be responsible for fixing the potential at the inert metal surface.

(5) Since organisms participate in the natural system, the system cannot be at equilibrium and hence the potential recorded is not a true equilibrium potential. The whole concept of a single oxidation-reduction potential characteristic of a particular environment is thus in some doubt.

Problems (1) and (2) can be minimised by good electrode design and field technique. However, problems associated with the performance of the platinum electrode (3-5) are more intractable.

In 1946, ZoBell[140] suggested that the measurment of Eh values of sediments could prove to be a useful means of characterizing sediments, although such values would be descriptive rather than physiochemically exact. Since the publication of ZoBell's paper, many workers have measured sediment Eh values and have found that Eh has a descriptive value. In general, the measurement of Eh as an operational parameter has found favour amongst biologists. For instance, Baas-Becking et al.[141] showed that there is a correlation between Eh and the viability of bacterial cultures, and Pearson and Stanley[142] found that measurement of Eh values provided an easy and rapid method for recording the relative reducing effects of organic effluent on the sediments of a particular marine environment.

The precision of environmental Eh measurements tends to be

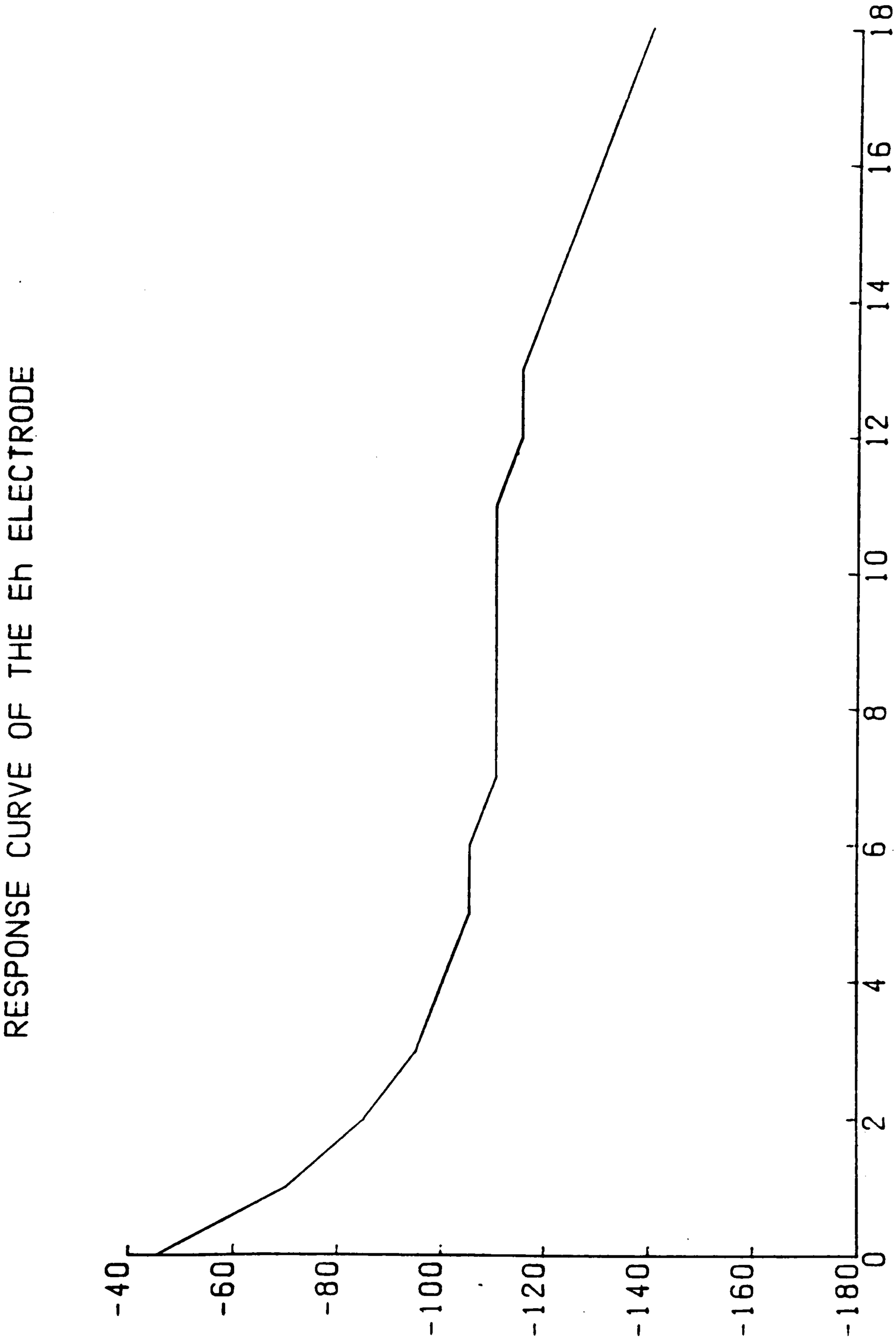
poor; Whitfield[119] has stated that precision is usually about ± 50 mV. However, as sediments exhibit a considerable range of Eh values (~700 mV) the imprecision of measurement represents only a small fraction of the total span of values found. On these grounds it seems reasonable to conclude that Eh measurements of sediments do have value, although Eh can only be a semi-quantitative parameter.

As part of this project, Eh values of sediments from many locations were measured. Originally this work was undertaken as the results of previous workers had suggested that methylmercury levels in sediments may be dependant upon the prevailing redox situation within sediments. However, it has been reported that both mercury methylation and demethylation can occur over a wide range of redox conditions[99,143,144]; it even has been reported that positive Eh values enhance the rates of both methylation and demethylation[59,99].

Method used in this Project for the Determination of Eh values of Sediments

An Orion Model 96-78 redox electrode was used in conjunction with an Orion 407A meter to measure Eh values of sediments either in situ or on preserved samples in the laboratory. The Orion electrode consisted of a flat platinum disc and an integral reference electrode designed to match conventional calomel electrode performance. Eh measurements were made by carefully inserting the electrode into the sediment and monitoring the Eh of the sample until a steady reading was obtained, i.e. when the drift in reading was less than 1 mV min^{-1}. Generally, it was found that Eh readings drifted towards more negative values after the electrode had been inserted into the sediment, and that stabilisation occurred only after 5-10 minutes. A typical response curve for the redox electrode is shown in Fig. 24. Similar response curves have been reported by other workers[119,142,145,]. After a stable Eh reading

Fig. 24


RESPONSE CURVE OF THE Eh ELECTRODE
TIME (min.)
Eh (mV)
-40
-60
-80
-100
-120
-140
-160
-180
0
2
4
6
8
10
12
14
16
18

for a sediment had been recorded, any sediment adhering to the electrode was washed off with distilled water and the platinum sensor was cleaned by rubbing it with a fine abrasive cloth. Occasionly, the potential of the electrode was measured in ZoBell solution (0.003 mol dm^{-3} potassium ferricyanide, 0.003 mol dm^{-3} potassium ferrocyanide and 0.1 mol dm^{-3} potassium chloride - Eh value = +182 mV) to check the performance of the liquid junction; this was usually found to function normally.

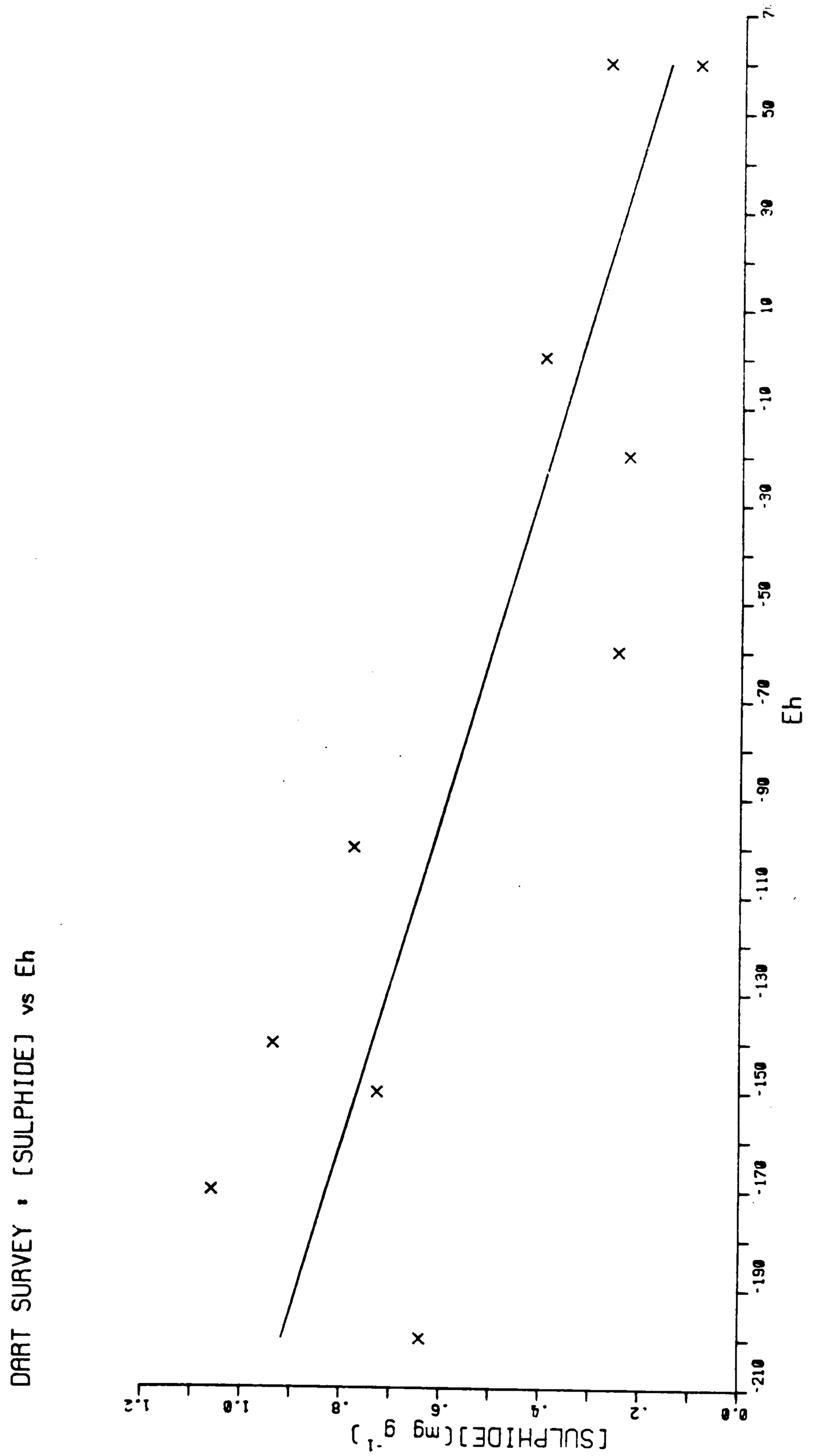
The Relationship Between Eh and Sulphide Content of Sediments

Previous workers[118,119] have reported correlations between Eh value and sulphide content for sediments with negative Eh values, i.e. sediments whose sulphide content was high enough to control the electrode potential. As part of this project both the Eh value and sulphide content of sediments from many locations were determined, and these correlations were limited. A major reason for this was the fact that Eh provides a measure of the ratio of oxidised to reduced species in a sediment, rather than a measure of their absolute concentrations. Thus a sandy or gravelly sediment containing a small amount of anoxic mud recorded a negative Eh value (normally associated with high sulphide levels) although the sulphide content of the sediment was found to be low. Additionally, it should be noted that a correlation between Eh and sulphide content is unlikely to be found in sediments with low sulphide concentrations as the reactions of other species would then control the electrode potential. However, significant correlations between these two parameters were found in locations where the matrix of all the sediments sampled was uniform; this is illustrated in Fig.25.

Conclusion

Although the measurement of environmental Eh values is

Fig. 25.


DART SURVEY - [SULPHIDE] vs Eh
[SULPHIDE](mg g-1)
Eh
0.0
.2
.4
.6
.8
1.0
1.2
70
50
30
10
-10
-30
-50
-70
-90
-110
-130
-150
-170
-190
-210

subject to a number of problems, previous workers have found that such measurements have descriptive value, and most agree that Eh is useful as a semi quantitative parameter.

In view of the problems associated with Eh measurements outlined in the introduction, and also because of the additional problems of (a) the drift in readings with time and (b) the inability of Eh measurements to indicate absolute concentrations of reduced species, it was decided that the determination of sulphide concentrations in sediments would provide a better measure of their degree of anoxicity. Therefore, in this thesis, correlations between methylmercury levels in sediments and the degree of anoxicity of the sediments are made primarily with reference to sulphide concentrations, although Eh values also are reported.

Finally, it should be noted that all Eh values reported in this thesis are potentials recorded by the combination redox electrode, based on calomel, and + 248 mV should be added to the measured values to correct them to the hydrogen scale. Both scales are commonly used in environmental work.

Chapter 10

The Determination of Organic Carbon Content of Sediments

The organic carbon content of sediment samples were determined using a Perkin Elmer Model 240 Elemental Analyser. This instrument accurately determines the carbon, hydrogen and nitrogen content of organic samples by detecting and measuring combustion products (CO_2, H_2O and N_2). Combustion occurs in pure oxygen under static conditions. The combustion products are then analysed automatically in a self-integrating, steady state, thermal conductivity analyser. Results are recorded in bar graph form on a 0-1 mV recorder.

Three systems in the Elemental Analyser perform the determinations. These are the combustion train, the analytical system and the electronics.

A small amount of sample (1-3 mg) is combusted in pure oxygen. A flow of helium then carries the combustion products over a series of catalysts which convert the carbon and nitrogen combustion products to CO_2 and N_2 respectively. The combustion products are then carried from the combustion train, through the analytical system, to atmosphere.

The analytical system consists essentially of adsorption traps and 3 pairs of thermal conductivity cells, arranged in series, for the detection, one pair each, of water, carbon dioxide and nitrogen. The platinum filaments of each cell pair are connected differentially in a bridge circuit so that any difference in the contents of the 2 cells will result in an electrical signal. A magnesium perchlorate trap between the first pair of cells adsorbs any water from the gas mixture before it enters the second cell so that the signal obtained form the corresponding bridge circuit is proportional to the amount of water

removed. Likewise, an adsorbing trap - containing a commercial preparation known as 'colocarb' - between the second pair of cells results in a signal in proportion to the carbon dioxide removed from the sample. The last pair of cells detects nitrogen by comparing the thermal conductivity of the remaining sample gas with that of pure helium.

Finally, the output signals from the bridge circuits are applied, through adjustable alternators, to the 1mV recorder.

The sensitivity of the method is as follows: carbon - 20 $\pm$ 5 uV/ug; hydrogen - 60 $\pm$ 16 uV/ug; nitrogen - 8 $\pm$ 2 uV/ug.

The precision of the method is very high with coefficients of variation of less than 1 % being recorded.

Chapter 11

Sampling and Storage Procedures

The importance of employing good sampling techniques and storage procedures for the collection and preservation of sediment samples prior to analysis is well understood. In particular, 3 factors need to be considered; namely, (1) the degree of homogeneity of the sample, (2) the depth of sediment at which the sample is taken and (3) the conditions and period of storage of the sample prior to analysis. These factors are discussed below.

Sampling Procedure

For this project, the collection of uniform, homogeneous samples was desirable as sub-samples of substantially different weights were required for the various analyses undertaken. For example, the method employed for the determination of sulphide required as little as 0.2 g of sediment, whereas ~5 g of sediment were required for the method of methylmercury determination. Difficulties were experienced in taking representitive sub-samples from sediments that were non-uniform in composition - e.g. sediments containing a mixture of silt, gravel and vegetation. Most of the sediments collected for analysis in this project were uniform and homogeneous, although it was not possible to obtain such sediments from all the locations sampled. When non-uniform sediments were the only type available from a particular location, sub-samples were taken from one component of the sediment (usually silt). In this way, sub-samples of similar composition were analysed and valid correlations between the concentrations of the species determined could be made; however, in these cases the values obtained for the concentrations of the species determined were not representitive of the samples as a whole.

It was important to consider the depth of sediment at which samples were taken for 2 reasons: (1) chemical and microbiological processes occuring within sediments change with depth, (2) concentrations of chemical species in sediments also may vary with depth, perhaps reflecting changes in pollution inputs to water systems over periods of time.

Considering (1): Surface layers of sediments generally are more aerobic - e.g. as shown by more positive Eh values - than sub-surface sediments and therefore lower concentrations of reduced chemical species are likely to be present in surface layers, e.g. sulphide which is oxidised by atmospheric oxygen to sulphate. Microbial species within sediments also change with variations in the degree of anoxicity, and hence depth, of sediments. However, the implication of this last point for the production of methylmercury in sediments is unclear. In experiments with marine sediments, Olsen and Cooper[59] found the net production of methylmercury to be greater under anaerobic conditions than under aerobic conditions, whereas in experiments with microbial reactors Bisogni and Lawrence[99] found the opposite, i.e., higher methylation rates under aerobic conditions.

To investigate further points (1) and (2), core samples were taken from the Mersey, Forth and Carron estuaries and analysed at 1" intervals for total mercury, methylmercury and sulphide content. Eh values were also measured. The results of these analyses are presented below.

<u>Mersey</u>

Depth (Inches)	(Sulphide) (mg g^{-1})	(MeHg) (ng g^{-1})	(Hg)TOT (ug g^{-1})	Eh (mV)
0 - 1	1.9	11.2	2.56	-180
3 - 4	1.2	10.8	2.45	-250
6 - 7	1.1	5.6	1.30	-280
9 - 10	1.3	4.3	1.40	-270
12 - 13	1.0	2.0	0.09	-280

Forth

Depth (Inches)	(Sulphide) (mg g^{-1})	(MeHg) (ng g^{-1})	(Hg)TOT (ug g^{-1})	Eh (mV)
0 - 1	2.8	2.8	1.06	-230
3 - 4	1.6	0.7	0.13	-280
6 - 7	1.5	0.5	0.12	-300
9 - 10	1.8	0.5	0.07	-300
12 - 13	1.3	0.5	0.05	-320

Carron

Depth (Inches)	(Sulphide) (mg g^{-1})	(MeHg) (ng g^{-1})	(Hg)TOT (ug g^{-1})	Eh (mV)
0 - 1	2.8	25.5	4.5	-220
3 - 4	1.7	58.6	5.5	-300
6 - 7	1.9	101.1	38.0	-380
9 - 10	1.7	126.6	36.8	-360
12 - 13	1.5	110.8	34.0	-380

For the Mersey and the Forth, decreases in both total mercury and methylmercury concentrations were found with increasing depth of sediment. The fall in total mercury concentrations may reflect changes in mercury input to the estuaries over time. The fall in methylmercury concentrations may be due to lower levels of total mercury in the deeper sediments and/or changes occurring in chemical and microbiological processes with increasing depth of sediment, resulting in lower methylation rates.

The core sample from the Carron estuary was taken near the outfall of a chemical plant. The marked increase in the

levels of total mercury with increasing depth of sediment indicates the presence of greater concentrations of effluent in the subsurface sediment. An increase in methylmercury levels with depth was also found although the rate of increase was not quite as high as that for total mercury and may have been influenced by the fall in sulphide concentration (see Chapters 19 and 20).

For all three estuaries, the highest concentrations of sulphide were found in the top 1" layer of sediment, although more negative Eh values were recorded at depth. This was an unexpected result, even though the surface-brown-oxic layer of the sediments was thin (~2mm thick). The result is probably due to the higher water content of the surface sediments: a high proportion of the sulphide content found in sediments is present in the interstitial water, in the form of dissolved hydrogen sulphide, hydrosulphide ion and divalent sulphide ion. Thus, drier subsurface sediments may be expected to contain lower concentrations of sulphide owing to their lower interstitial water content.

Conclusions

Theoretical considerations, outlined at the beginning of this chapter, suggested that different concentrations of chemical species would be found at various depths of sediment, and the analysis of core samples taken from 3 estuaries indicated this to be true.

From most locations sampled in this project, sediments of a uniform, homogeneous matrix were obtained. For those sampling stations where the surface oxic layer of sediment was more than 1 cm thick, samples of the oxic sediment and the underlying anoxic sediment were collected seperately. However, the oxic layer of the sediments of most of the locations sampled was too thin for separate sampling to be possible, and only 1 sample, taken from the top 5 cm of sediment, was collected from these locations, this being the

depth of sediment most likely to contain the highest concentrations of the species of interest in this project. All samples were placed directly into polythene bottles which were then closed with gas-tight caps to prevent exposure of the sediment to air.

Storage Procedures

The principal aim of the environmental work undertaken in this project was to determine in situ values for concentrations of total mercury, methylmercury and sulphide in sediments and investigate correlations between these parameters. At the outset of the project it was realised that it would not be possible to analyse all samples within a short period after collection, and that storage of some samples prior to analysis would be necessary. The question of possible changes occurring in the concentrations of chemical species in the sediments during storage then arose. Previous work had shown that total mercury levels are not affected by storage temperatures up to 15°C[51]. However, it was also known from previous work that methylmercury levels in sediments are liable to change substantially during storage at ambient temperatures[146]. Sulphide has also been reported as being labile in the sediment environment[109].

Experiments were designed, therefore, to determine if freezing sediment samples immediately after collection, followed by storage of the samples in deep freeze, would preserve in situ levels of methylmercury and sulphide. The experimental design is illustrated below by reference to the method employed to ascertain possible changes in methylmercury levels in sediments during cold storage.

Collection of Samples

Five sediment samples were collected at low water from intertidal sites in the Carron estuary, Lothian, Scotland.

The samples were transported quickly to the laboratory where sub-samples were analysed for methylmercury content. The analysis of the sub-samples was commenced as soon as possible after the collection of the sediments (~20 minutes) in order to obtain in situ values for levels of methylmercury. The remainder of the 5 samples were then frozen using dry ice and kept in deep freeze for a period of 7 days before being re-analysed for methylmercury content.

Results and Analysis of Data

The results obtained for the levels of methylmercury in the sediments, in situ and after 7 day's storage in deep freeze, are presented below.

Sample No.	In Situ	Frozen	Difference
1	38.2 ng g^{-1}	32.9 ng g^{-1}	5.3 ng g^{-1}
2	30.5 " "	32.6 " "	-2.6 " "
3	46.1 " "	45.8 " "	0.3 " "
4	42.8 " "	38.3 " "	4.5 " "
5	32.3 " "	36.2 " "	-3.9 " "

In order to decide whether there has been any real change in the concentrations of methylmercury in the sediments during storage, a statistical test, known as Student's t-test, can be applied to determine whether the discrepancies between the concentrations of methylmercury in the in situ and stored samples is "significant". If no real change has occurred in the methylmercury content of the sediments during storage, and the observed discrepancies are due soley to lack of precision in the analytical method, then the average discrepancy should not differ significantly from zero. The test statistic used can be written as follows:

$$t = \frac{\bar{d} - 0}{\sqrt{s^2 d/n}}$$

where $\bar{d}$ is the average discrepancy (0.72), Sd^2 is the

sample estimate of variance (16.952) and n the number of samples (5). It should be noted that the sample estimate of variance was calculated from (n-1) independant values of $(d-\bar{d})$ and is therefore said to be based on 4 degrees of freedom.

Calculations gave a value of 0.39 for student's t. From statistical tables it is found that for the t-distribution with 4 degrees of freedom, there is only a 5 % chance of $|t| > 2.78$, 1 % chance of $|t| > 4.60$ and 0.1 % chance of $|t| > 8.61$.

If the calculated value of t had exceded the value given by the 5 % probability level, the difference in the 2 sets of results would have been regarded as probably significant. If the 1 % probability level had been exceded, the discrepancies would have been regarded as definitely significant, and if the 0.1 % probability level had been exceded, the discrepancies would have been considered highly significant. For a more detailed account of Student's t-test and other statistical methods, see references[147,148].

The calculated value of t, 0.39, falls below the 5 % critical value, and thus the data gives no reason to suppose that there has been any change in the methylmercury concentration of the sediments during 7 days storage in deep freeze.

Similar tests, performed on sediments from various locations, revealed that no significant change occurs in the methylmercury and sulphide concentrations of sediments during (a) 24 hours storage at ambient temperature and (b) 7 days storage in deep freeze. The Eh values of sediments were also found to be unaffected by these storage conditions. Interestingly, using the same tests, the sulphide contents of sediments collected from the Dart and Teign estuaries, S.W. England, were found to be stable on storage at ambient temperature for at least 5 days. This result suggests

that sulphide might be more stable in the sediment environment than has been suggested previously[109]. However, highly significant differences were found between in situ methylmercury contents of sediments and methylmercury levels in sediments after storage at ambient temperature for 7 days. In this case a steady increase in the methylmercury contents of the sediments, up to concentrations approximately 50 % greater than the in situ values, occurred over a period of 14 days; this was then followed by a steady decline in the methylmercury levels in the sediments. This "growth and decay" effect has been reported elsewhere-(102,103,146).

Conclusions

Statistical tests showed that in situ values for methylmercury and sulphide contents of sediments could be obtained from the analysis of sediment samples which had been stored in deep freeze for up to 7 days, or at ambient temperature for up to 24 hours. Therefore, during the course of this project, sediment samples either were kept at ambient temperature and analysed for methylmercury and sulphide content within 24 hours of sampling, or they were frozen immediately after collection and analysed within 7 days.

SECTION 3

ENVIRONMENTAL WORK

Chapter 12

Mercury in Sediments of South West England Estuaries

Introduction

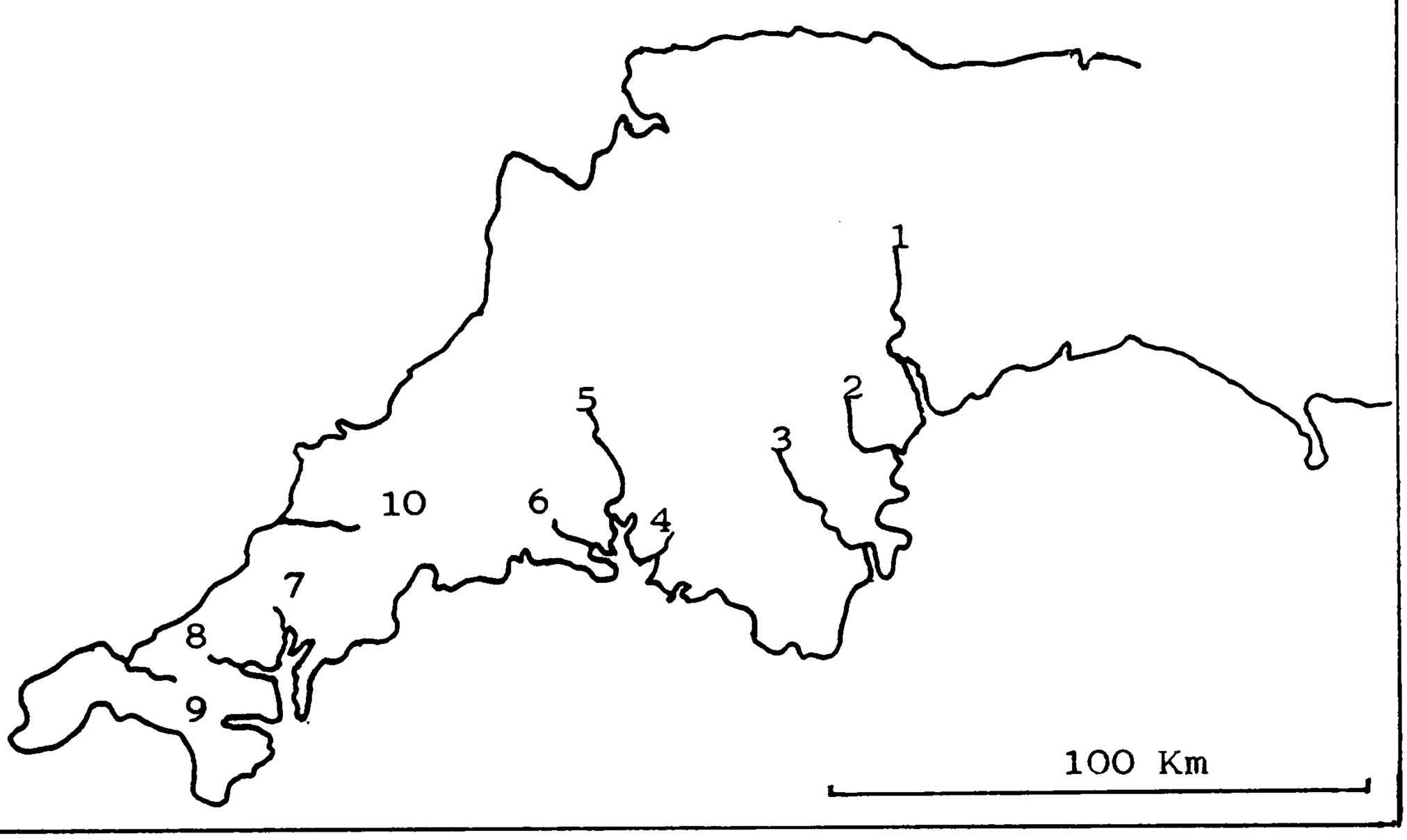
Morton[102] and Bartlett[103] have reported methylmercury levels in sediments of two polluted U.K. estuaries (the Mersey and Clyde). However, no studies have been made on methylmercury levels in sediments of relatively unpolluted U.K. estuaries, and indeed, few studies of methylmercury levels in relatively unpolluted sediments have been made world wide. Little general information is known, therefore, concerning relationships between methylmercury levels, total mercury levels and other sediment parameters in relatively clean sediments. Surveys of the comparatively clean estuaries of South West England were undertaken to investigate the relationships between various parameters in clean sediments, and to compare these with similar relationships observed in polluted sediments.

Ten estuaries in South West England were selected for survey. These were the Exe, Teign, Dart, Plym, Lynher, Truro, Restronguet, Hayle and Gannel (locations are shown in Fig. 26). These estuaries receive small pollution inputs from agricultural, domestic and industrial sources, but the quality of the water of these estuaries generally is good. The estuaries also drain mineralised catchment areas, where until the end of the century metals, including arsenic, copper, lead, tin and zinc were mined; there are, however, no significant mercury sources in these catchment areas[149].

A few total mercury levels in the sediments of these estuaries had been reported before the current work was undertaken[150], and relatively low levels (<1.0 ug g^{-1}) had been found; although subsequently, other workers have reported high sediment mercury concentrations in certain areas of the Plym (2.6 ug g^{-1})[151] and Lynher (2.1 ug g^{-1})[152].

Fig. 26.

Map Showing Location of Estuaries in S.W. England



Key:-

1 : Exe
2 : Teign
3 : Dart
4 : Plym
5 : Tamar
6 : Lynher
7 : Truro
8 : Restronguet
9 : Hayle
10 : Gannel

Sampling and Analysis

Two surveys of the South West England estuaries were undertaken, with the assistance of the I.C.I. Brixham Laboratory, Devon, during the periods June - August 1981, and October 1982. Sediment samples were collected from intertidal locations at low water, following the procedure described in Chapter 11. Samples collected during the 1981 survey were kept at ambient temperature and analysed for methylmercury and sulphide content within 24 hours of collection. The samples were then stored under refridgeration until they could be analysed for total mercury content (up to 2 months). Sediments collected during the 1982 survey were frozen with solid CO_2 immediately after collection, and then stored in deep freeze until analysis for methylmercury and sulphide content (up to 1 week). The sediments were then stored under refridgeration and analysed for total mercury content at a later date (up to 1 month).

Sediments collected during the first survey were analysed for sulphide content using the direct iodometric method (see Chapter 6). As was noted previously, many reduced species present in sediments are interferents for this method. However, the proness of the method to interferents was the rationale for using it: it was hoped that the method would provide a general measurement of the degree of anoxicity of the sediments in addition to estimating their sulphide contents. This approach was abandoned after the first survey, and subsequently, both the Eh values and sulphide contents of the sediments were determined separately, the specific potentiometric method being the prefered method of sulphide determination.

Results

The results of the 1981 survey are presented in Table 9. Compared to previously reported total mercury and methylmercury levels in sediments of the mercury-polluted Mersey

Estuary	Map No.	O.S. Ref.	Sediment Type	Sulphide (mg g^{-1})	Me Hg (ng g^{-1})	Tot. Hg (ug g^{-1})
Exe	192	987/800	Gravel with some mud	0.1	<0.5	<0.05
"	192	974/844	Black mud	0.7	1.6	0.06
"	192	979/819	Black mud	1.4	0.9	0.13
"	192	967/875	Black mud	2.4	2.2	0.25
"	192	986/836	Black mud	1.0	1.0	0.08
Dart	202	806/602	Black mud	1.5	0.6	0.52
"	202	847/567	Brown mud	1.0	1.2	0.33
"	202	847/567	Watery brown mud	1.6	3.7	0.37
"	202	869/548	Black mud with some gravel	1.7	3.8	1.56
"	202	863/521	Brown/black mud with some gravel	1.6	1.4	4.46
Plym	201	503/544	Brown /black mud	1.2	2.4	0.26
"	201	503/553	Brown/black mud	1.2	3.0	0.35
"	201	520/562	Black mud	0.9	4.0	0.14
"	201	505/554	Gravel	0.1	0.1	0.08
Tamar	201	433/564	Black mud	1.6	1.2	0.48
"	201	426/577	Gravel	<0.1	<0.5	0.07
"	201	441/549	Black mud	2.2	1.7	0.33
"	201	432/637	Black mud	2.5	1.3	0.66
"	201	422/649	Black mud	2.1	1.0	0.46
Lynher	201	417/566	Watery black mud	2.0	2.5	0.82
"	201	364/572	Watery black mud	2.0	1.6	0.83
"	201	397/561	Watery black mud	2.5	3.0	0.63
"	201	407/570	Watery black mud	1.8	1.5	0.61

Table 9

Sediment Sample Analyses, S.W. England Estuaries

cont'd/...

Estuary	Map No.	O.S. Ref.	Sediment Type	Sulphide (mg g^{-1})	Me Hg (ng g^{-1})	Tot. Hg (ug g^{-1})
cont'd/....						
Restronguet	204	803/387	Grey mud	4.2	3.5	0.34
"	204	791/393	Mud & decaying vegetation	3.2	3.0	0.45
"	204	813/386	Grey mud	2.4	2.9	0.41
"	204	817/372	Grey mud with some gravel	0.5	1.1	0.15
Truro	204	833/438	Black mud	1.4	1.6	0.39
"	204	835/430	Black mud	0.9	1.4	0.42
"	204	842/424	Black mud	1.4	1.7	0.30
"	204	847/416	Black mud	2.2	1.8	0.33
"	204	843/404	Gravel	0.4	<0.4	0.16
Teign	192	877/722	Grey mud with gravel	0.7	3.6	0.08
"	192	903/724	Grey mud with gravel	1.2	3.3	0.16
"	192	926/724	Brown/grey mud	0.4	3.2	0.67
"	192	930/729	Black mud with gravel	0.6	2.9	0.11
"	192	893/723	Brown/black mud with gravel	1.5	2.7	0.14
Hayle	203	547/364	Sand and shale	0.9	0.8	0.36
"	203	558/374	Brown mud	1.0	0.8	0.32
"	203	563/378	Black mud	1.6	0.6	0.26
Gannel	200	793/609	Sand	0.4	0.4	<0.05
"	200	804/607	Sand with some brown mud	0.6	0.4	0.20
"	200	809/606	Sand	0.7	0.4	<0.05
"	200	813/608	Clay	1.4	0.5	0.07

Table 9 cont'd

estuary (average total mercury content = 1.2 ug g^{-1}, average methylmercury content = 6.5 ng g^{-1})[103], the levels of species reported in Table 9 are generally low.

Plots of methylmercury conc. vs. total mercury conc., and methylmercury conc. vs. sulphide conc. for the data presented in Table 9 are shown in Figs. 27 and 28 respectively. A least squares analysis of the data represented in Fig. 27 demonstrates the absence of a linear relationship between total mercury and methylmercury levels ($r = 0.1$, $P > 0.1$). Conversely, a least squares analysis of the data represented in Fig. 28 demonstrates the existance of a significant linear relationship between methylmercury and sulphide levels; the equation and correlation coefficient describing the relationship are shown below.

$$[MeHg]\,(ng\ g^{-1}) = 0.54\,[Sulphide]\,(mg\ g^{-1}) + 1.02$$

$$S.D. = 1.06, \quad r = 0.40 \ (P<0.01)$$

The results of the 1981 survey had shown that of the ten estuaries examined, sediments of the Dart, Plym and Teign estuaries contained the highest levels of mercury species, and these estuaries were selected for further investigation. The results of the second survey are presented in Table 10.

A least squares analysis of the sulphide, methylmercury and total mercury data presented in Table 10 demonstrates significant correlations for the following relationships:-

<u>Plym</u>

$$[MeHg]\,(ng\ g^{-1}) = 7.08\,[Hg]_{TOT}\,(ug\ g^{-1}) + 0.01 \ (Fig\ 29)$$

$$S.D. = 0.68, \quad r = 0.78 \ (P<0.05)$$

$$[MeHg]\,(ng\ g^{-1}) = 1.71\,[Sulphide]\,(mg\ g^{-1}) + 0.75 \ (Fig.\ 30)$$

$$S.D. = 0.34, \quad r = 0.95 \ (P<0.001)$$

PLYM SURVEY 13.10.82

SAMPLING STATION	Eh	[SULPHIDE]	[MeHg]	$[Hg]_{TOT}$
1	+ 40 mV	0.59 mg g^{-1}	2.4 ng g^{-1}	0.34 µg g^{-1}
2	- 90 mV	0.64 mg g^{-1}	2.0 ng g^{-1}	0.30 µg g^{-1}
3	- 15 mV	1.88 mg g^{-1}	3.8 ng g^{-1}	0.44 µg g^{-1}
4	0 mV	0.38 mg g^{-1}	1.2 ng g^{-1}	0.26 µg g^{-1}
5	- 30 mV	0.21 mg g^{-1}	1.0 ng g^{-1}	0.28 µg g^{-1}
6	- 60 mV	0.36 mg g^{-1}	1.5 ng g^{-1}	0.18 µg g^{-1}
7	-290 mV	0.91 mg g^{-1}	2.2 ng g^{-1}	0.18 µg g^{-1}
8	-130 mV	0.14 mg g^{-1}	0.6 ng g^{-1}	0.08 µg g^{-1}

TEIGN SURVEY 14.10.82

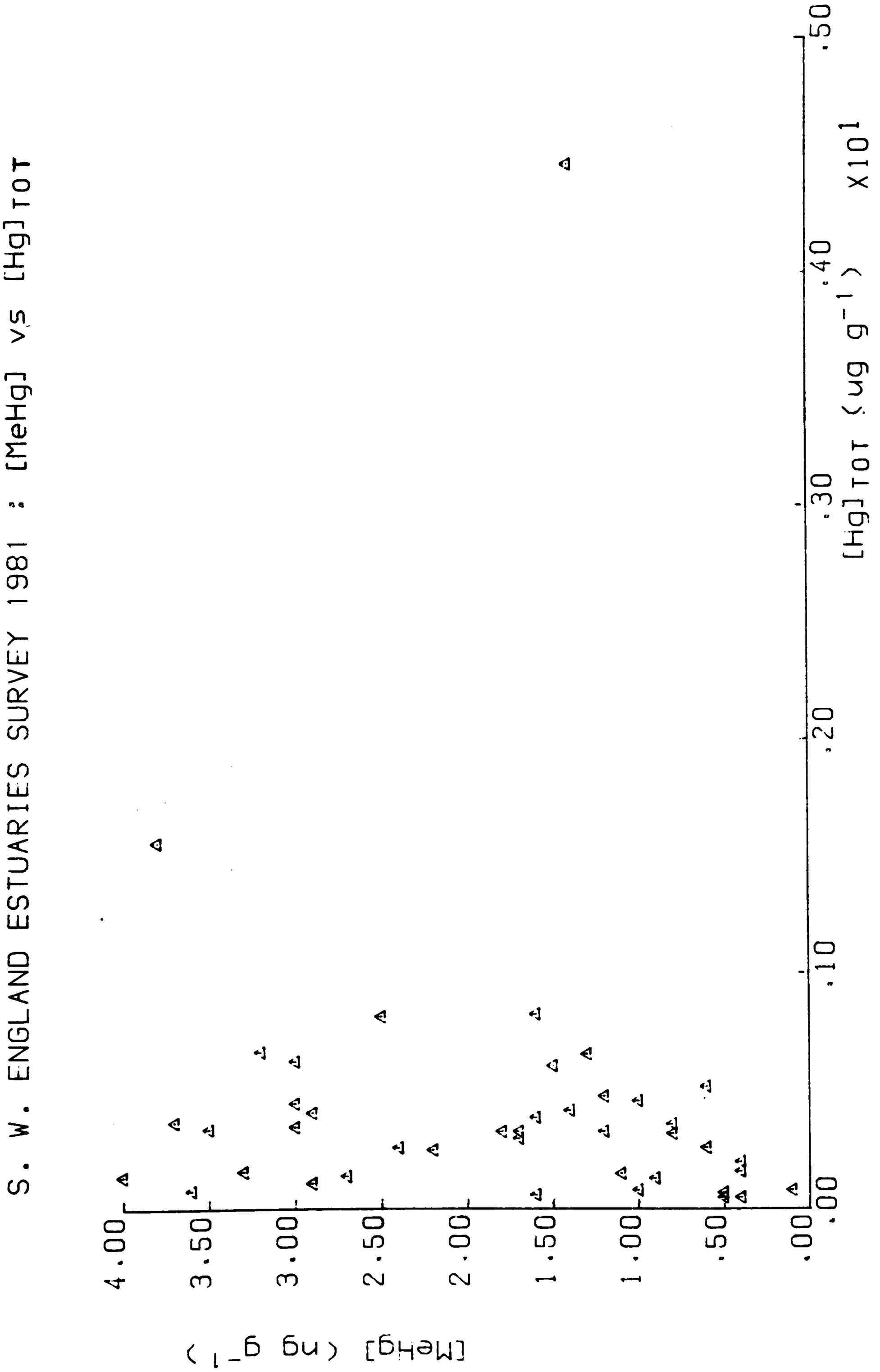
SAMPLING STATION	Eh	[SULPHIDE]	[MeHg]	$[Hg]_{TOT}$
1	- 70 mV	0.35 mg g^{-1}	1.1 ng g^{-1}	0.21 µg g^{-1}
2	+ 60 mV	0.17 mg g^{-1}	0.5 ng g^{-1}	0.23 µg g^{-1}
3	- 20 mV	0.83 mg g^{-1}	2.3 ng g^{-1}	0.34 µg g^{-1}
4	- 90 mV	0.69 mg g^{-1}	3.2 ng g^{-1}	0.13 µg g^{-1}
5	+ 50 mV	0.01 mg g^{-1}	<0.5 ng g^{-1}	0.33 µg g^{-1}
6	-110 mV	0.77 mg g^{-1}	1.4 ng g^{-1}	0.15 µg g^{-1}
7	- 90 mV	1.70 mg g^{-1}	3.0 ng g^{-1}	0.19 µg g^{-1}
8	-180 mV	1.47 mg g^{-1}	1.4 ng g^{-1}	0.09 µg g^{-1}

DART SURVEY 14.10.82

SAMPLING STATION	Eh	[SULPHIDE]	[MeHg]	$[Hg]_{TOT}$
1	- 70 mV	0.52 mg g^{-1}	2.8 ng g^{-1}	1.72 µg g^{-1}
2	-130 mV	0.21 mg g^{-1}	1.0 ng g^{-1}	0.39 µg g^{-1}
3	- 90 mV	0.73 mg g^{-1}	2.7 ng g^{-1}	0.46 µg g^{-1}
4	+ 10 mV	<0.01 mg g^{-1}	0.5 ng g^{-1}	0.11 µg g^{-1}
5	-170 mV	1.06 mg g^{-1}	3.1 ng g^{-1}	0.38 µg g^{-1}
6	-160 mV	2.04 mg g^{-1}	3.5 ng g^{-1}	0.32 µg g^{-1}
7	-210 mV	0.97 mg g^{-1}	2.5 ng g^{-1}	0.26 µg g^{-1}
8	-220 mV	0.76 mg g^{-1}	2.3 ng g^{-1}	0.29 µg g^{-1}

Table 10 - Sediment Sample Analyses, S.W. England Estuaries

Fig. 27.


S. W. ENGLAND ESTUARIES SURVEY 1981 : [MeHg] vs [Hg]TOT
[MeHg] (ng g-1)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
.50
.00
.00
.10
.20
.30
.40
.50
[Hg]TOT (ug g-1) X101

S. W. ENGLAND ESTUARIES SURVEY 1981 : [MeHg] vs [SULPHIDE]

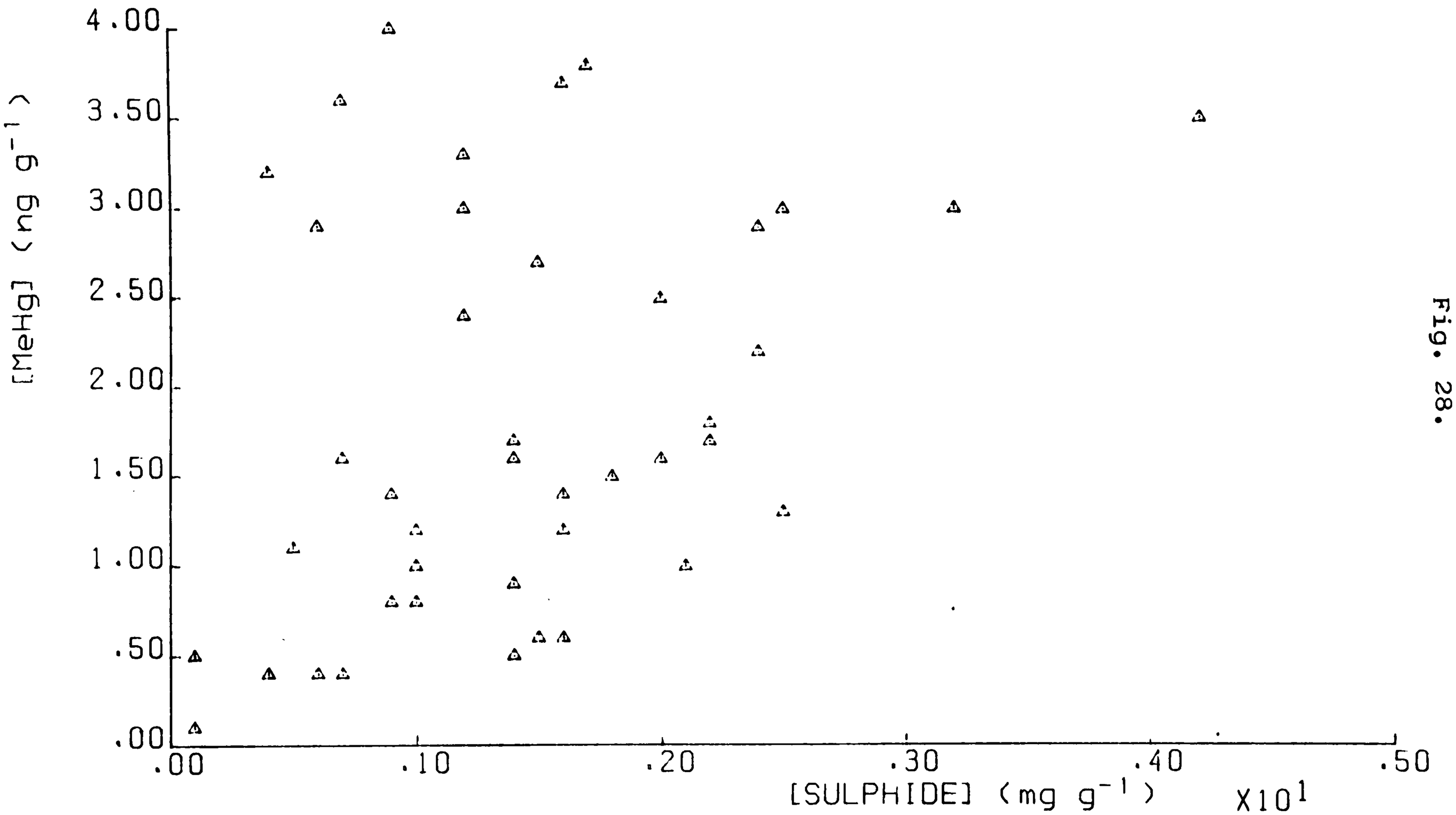


Fig. 28.

Fig. 29

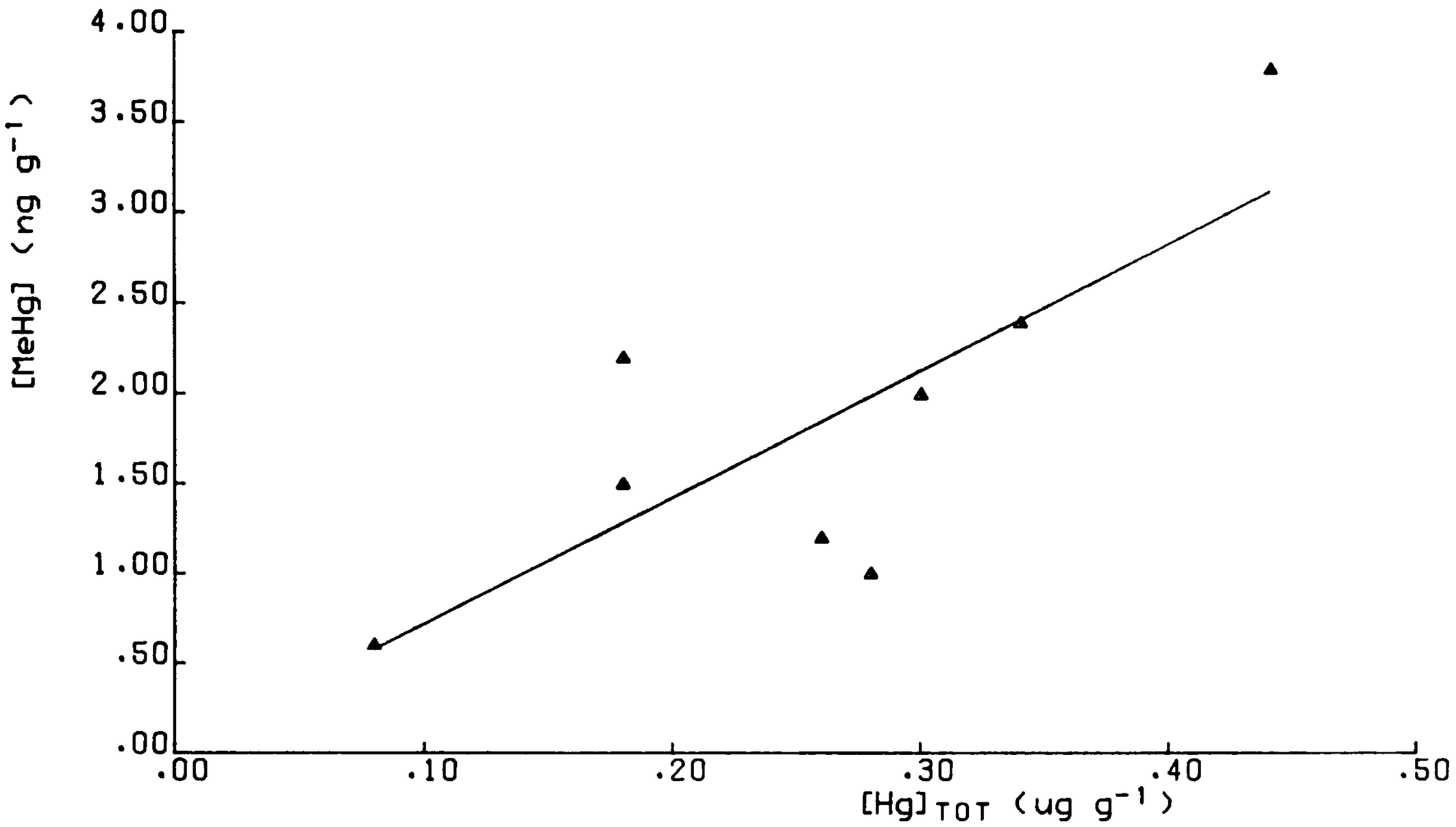

PLYM SURVEY 1982 : [MeHg] vs [Hg]TOT
[MeHg] (ng g-1)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
.50
.00
.00
.10
.20
.30
.40
.50
[Hg]TOT (ug g-1)

Fig. 30

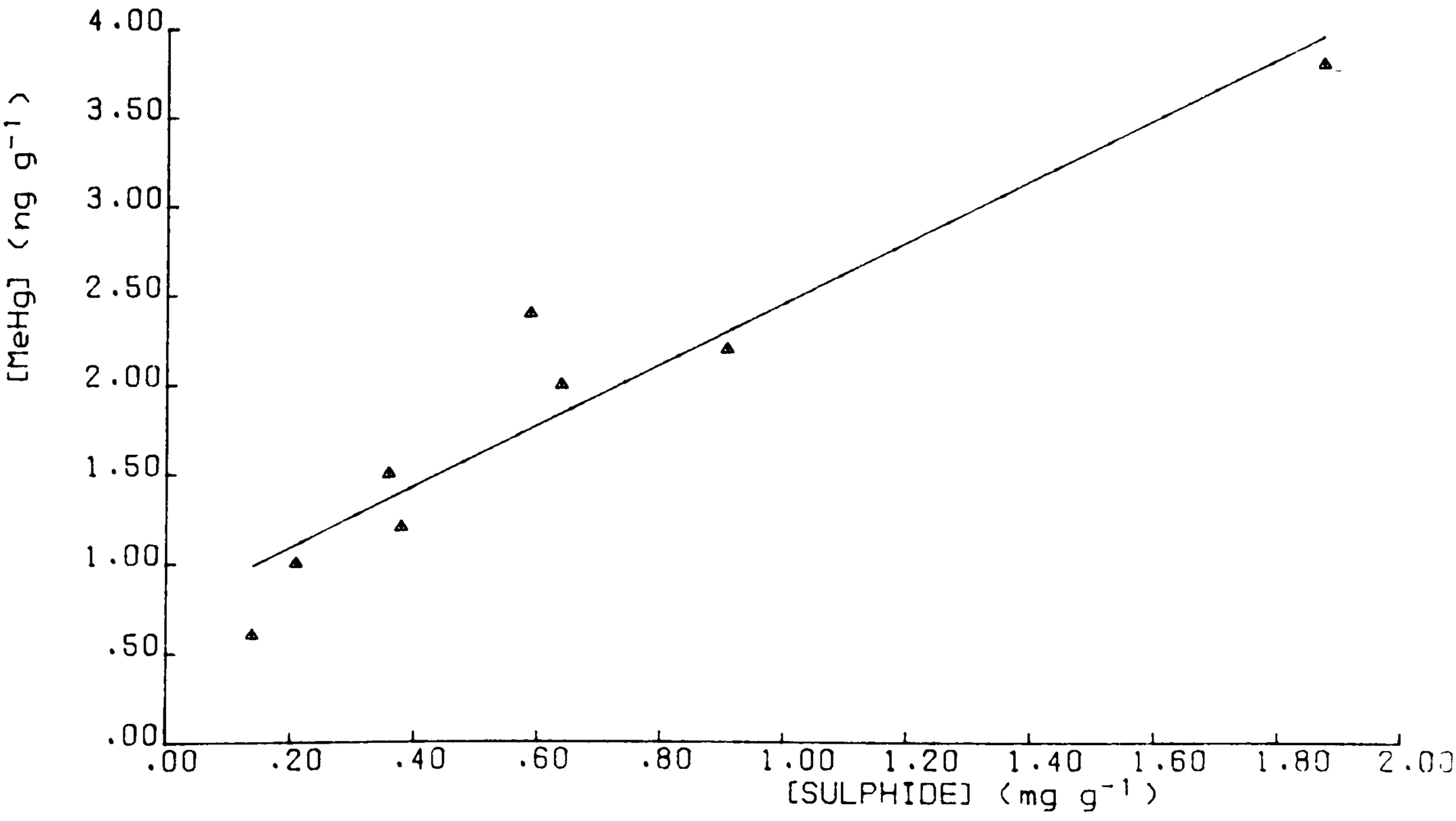

PLYM SURVEY 1982 : [MeHg] vs [SULPHIDE]
[MeHg] (ng g-1)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
.50
.00
.00
.20
.40
.60
.80
1.00
1.20
1.40
1.60
1.80
2.00
[SULPHIDE] (mg g-1)

Fig. 31.

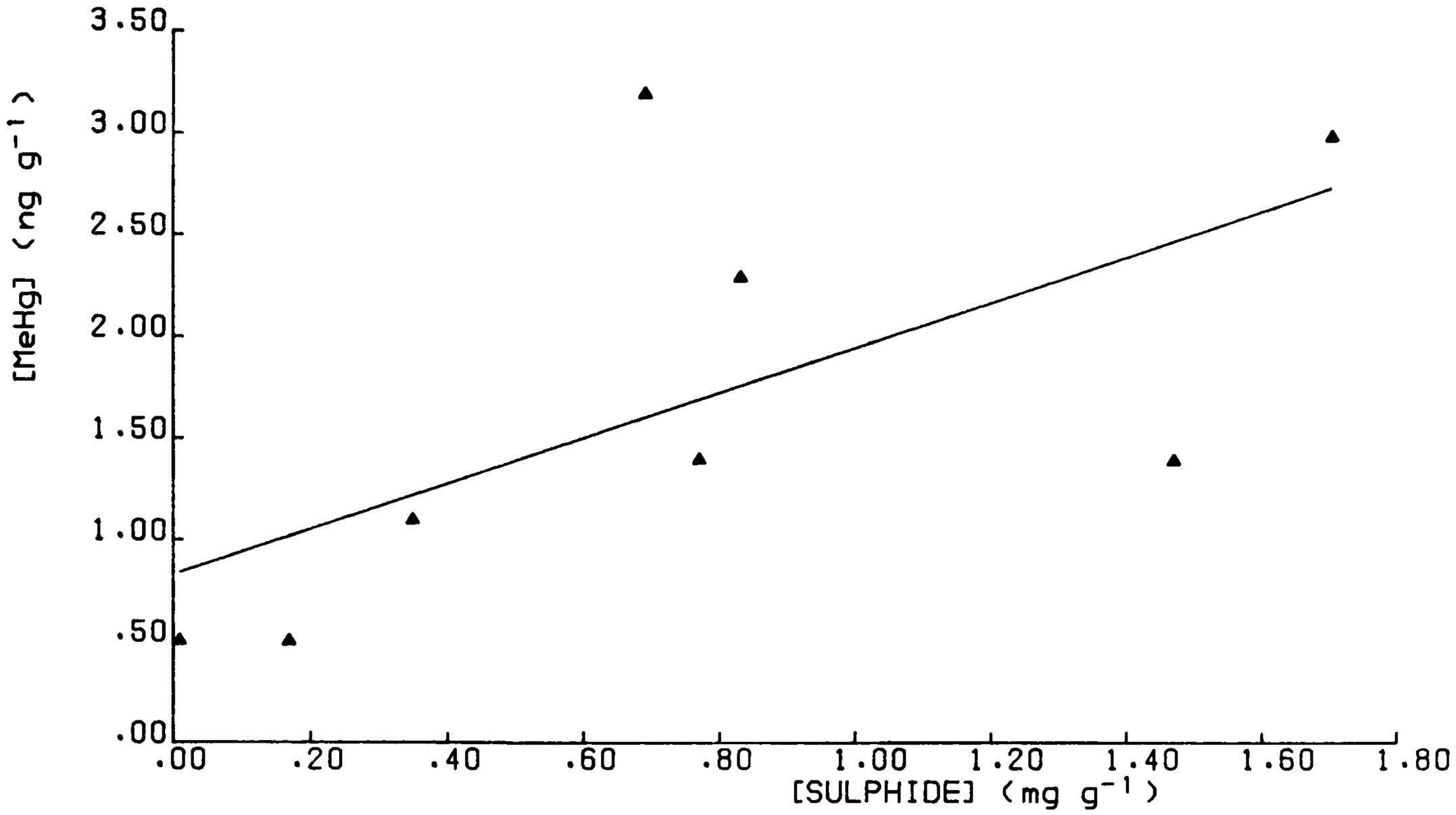
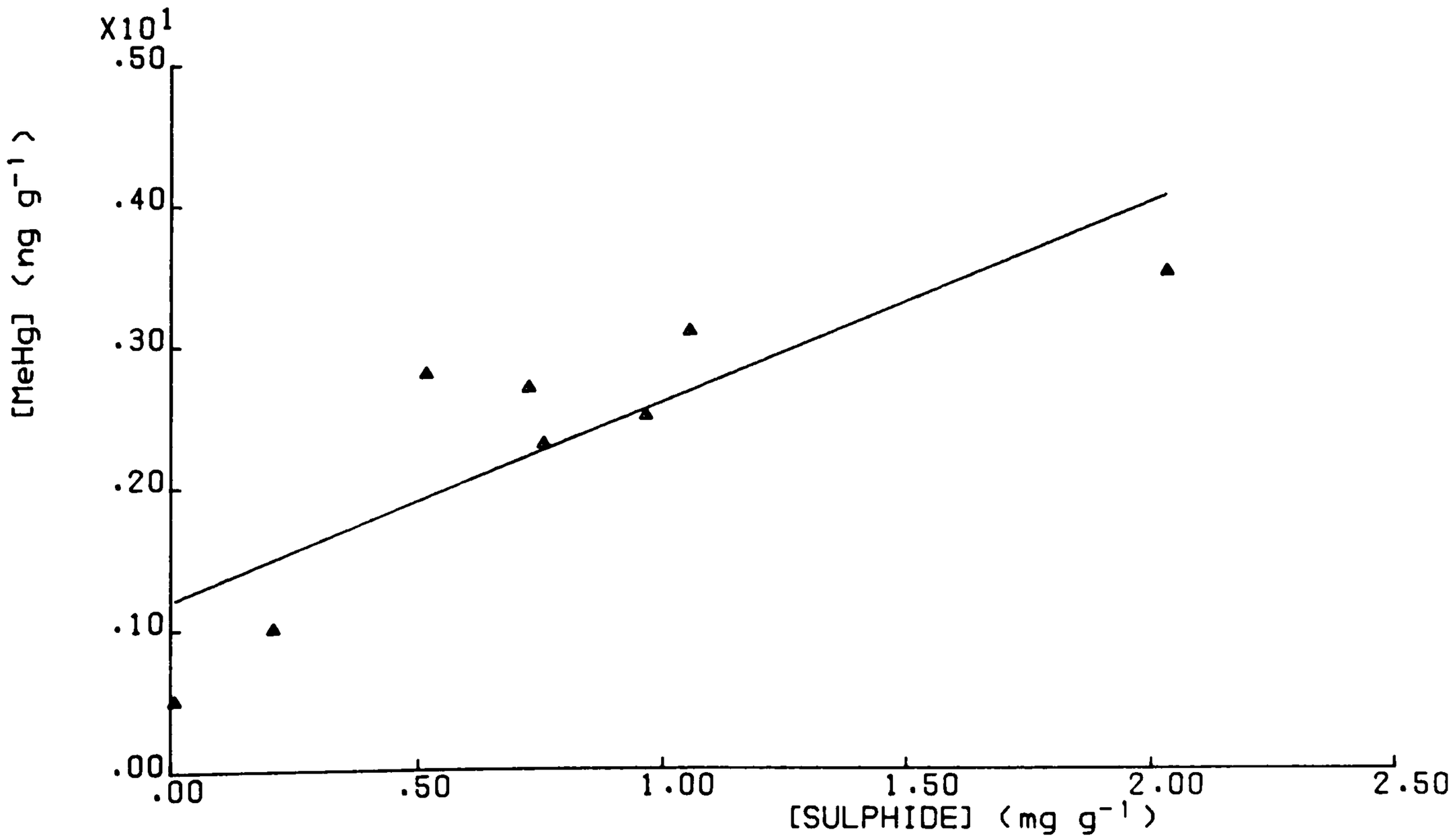

TEIGN SURVEY 1982 : [MeHg] vs [SULPHIDE]
[MeHg] (ng g-1)
3.50
3.00
2.50
2.00
1.50
1.00
.50
.00
.00
.20
.40
.60
.80
1.00
1.20
1.40
1.60
1.80
[SULPHIDE] (mg g-1)

Fig. 32.


DART SURVEY 1982 : [MeHg] vs [SULPHIDE]
X10 1
[MeHg] (ng g-1)
.50
.40
.30
.20
.10
.00
.00
.50
1.00
1.50
2.00
2.50
[SULPHIDE] (mg g-1)

Teign

$$[MeHg]\,(ng\ g^{-1}) = 0.83\,[Sulphide]\,(mg\ g^{-1}) + 1.13 \quad \text{(Fig. 31)}$$

$$S.D. = 0.87, \quad r = 0.64\ (p<0.1)$$

Dart

$$[MeHg]\,(ng\ g^{-1}) = 1.39\,[Sulphide]\,(mg\ g^{-1}) + 1.20 \quad \text{(Fig. 32)}$$

$$S.D. = 0.61, \quad r = 0.84\ (P<0.01)$$

No significant correlations were found between methylmercury and total mercury levels in sediments of the Teign and Dart, or methylmercury levels and Eh values in sediments of the Dart and Plym.

Discussion

These results support observations reported previously, that anaerobic conditions are more favourable to methylmercury production than aerobic conditions[59]. The results also show that for relatively unpolluted estuarine sediments, sulphide concentration is a more important factor than total mercury concentration in controlling methylmercury levels. The relationship between sediment methylmercury and sulphide levels is discussed in Chapter 19.

No relationship was found between sediment methylmercury levels and Eh values in samples collected from the Plym and Dart estuaries. A significant correlation between these two parameters may have been expected as Eh values of sediments are normally related to sulphide concentration. However, only the Teign data showed a significant correlation between the two parameters ($r = -0.76$, $P<0.05$). As was noted in Chapter 9, Eh measurements tend to be imprecise and thus are often unreliable. The results reported here suggest that the determination of sulphide concentration may provide a better measurement of the degree of anoxicity of sediments than measurement of Eh values.

Chapter 13

Mercury in River Carron Sediments

Description of the River

The River Carron flows through the Lothian region, Scotland, and joins the Firth of Forth at Grangemouth (Fig. 33). It is a polluted river having suffered from urban and industrial waste emission. In the upper reaches of the river there is an input from a drinking water treatment works. There are several sewage inputs in to the middle and lower reaches of the river, along with a discharge from a waste disposal plant and a discharge from a paper mill, the river having suffered badly, in particular, from the latter[153]. There is also an input from the Forth and Clyde Canal; the canal receives an input from an aluminium plant which is high in nitrate content and also an occasional source of oil. However, the most significant source of mercury in the Carron is the effluent of a dye works, which is discharged in to the lower reaches of the river.

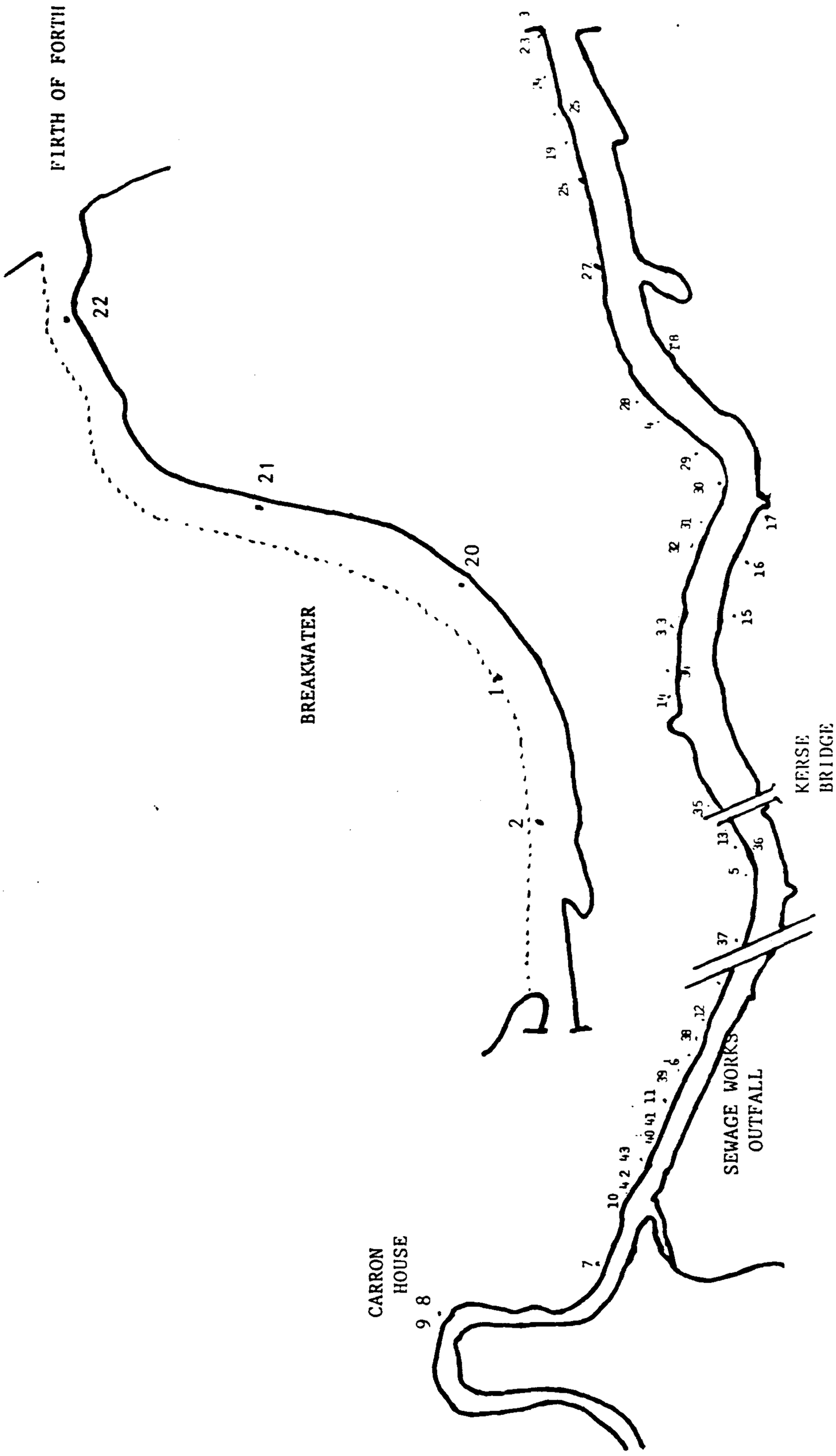
The average flow of the Carron varies from 11.6 to 1.1 $m^3 s^{-1}$ during the year, the rate being at a maximum in November and at a minimum in August.[154].

Sampling and Analysis

Three surveys of the Carron were undertaken during the period November 1981, July 1982 and November 1982. Sediment samples were collected at low water from the intertidal sediment zone of the river stretching from the breakwater to Carron House (Fig. 33). The samples collected in November 1981 were analysed at I.C.I. Ltd., Grangemouth, Lothian, Scotland, for sulphide and methylmercury content within a short time after collection; the total mercury content of

Fig. 33.

Map of the Carron Estuary



the samples was determined later at Leicester. Samples collected in July and November 1982 were frozen with dry ice immediately after collection and transported frozen to Leicester, where they were kept in deep freeze until analysis for sulphide and methylmercury content (up to 1 week). Eh values of the sediments were measured in situ. Before these surveys were undertaken, no data for methylmercury and total mercury levels in Carron sediments had been published.

Results

The results of the surveys are presented in Tables 11 to 13. The most interesting feature of the data is the relationship between methylmercury and sulphide levels.

A plot of the methylmercury and sulphide concentrations obtained from the preliminary 1981 survey (Fig. 34) shows that methylmercury concentrations rise initially with increase in sulphide concentration, but that after a concentration between 1.0-2.0 mg g^{-1} sulphide is exceded, methylmercury levels decay with further increase in sulphide concentration. No correlation between methylmercury and total mercury concentrations was found.

The results of the more extensive July 1982 survey demonstrate a linear relationship between methylmercury and sulphide levels up to a sulphide concentration of 1.4 mg g^{-1} (Fig. 35). A least squares analysis of the data points up to 1.4 mg g^{-1} sulphide gave a linear correlation coefficient (r) of 0.84 ($P<0.001$) and the following equation for the straight line:

$$[\text{Me Hg}]\,(\text{ng g}^{-1}) = 33.24\,[\text{Sulphide}]\,(\text{mg g}^{-1}) + 8.30,$$

$$\text{standard deviation (S.D.)} = 7.21$$

For this survey, only one sample was found to contain a sufficiently high sulphide concentration to present evidence of a maximum point in the graph. A poor linear

Table 11

Carron Survey - November 1981

Sample No.	Sulphide (mg g^{-1})	MeHg (ng g^{-1})	[Hg] TOT (ug g^{-1})
1	0.48	< 0.5	1.09
2	2.05	36.9	3.13
3	0.56	< 0.5	0.05
4	2.44	2.9	2.51
5	0.63	8.4	1.06
6	2.49	29.8	2.45
7	1.07	44.1	2.34
8	0.94	45.6	1.73

Table 12

Carron Survey - July 1982

Sample No.	Sulphide (mg g^{-1})	MeHg (ng g^{-1})	Hg TOT (ug g^{-1})
9	0.78	36.1	3.14
10	0.73	32.9	3.60
11	0.73	44.2	3.13
12	1.40	46.9	3.25
13	0.60	27.7	3.52
14	0.72	36.1	3.33
15	0.92	39.7	2.36
16	0.47	25.2	3.91
17	0.51	36.9	3.42
18	1.05	38.3	3.48
19	0.70	32.9	3.37
20	2.44	12.9	3.39
21	0.34	10.3	2.85
22	0.15	3.1	1.90

Table 13

Carron Survey - November 1982

Sample No.	Eh (mv)	Sulphide (mg g^{-1})	MeHg (ng g^{-1})	TOC (%)	[Hg] TOT (ug g^{-1})
23	+ 60	0.08	2.8	5.91	1.11
24	- 80	0.60	26.1	4.39	2.85
25	-110	0.64	26.9	8.05	3.95
26	-110	0.69	18.9	7.61	3.70
27	- 80	0.58	24.6	6.23	1.00
28	0	0.90	42.1	8.01	1.99
29	- 25	0.77	28.5	3.78	2.63
30	-140	1.14	42.2	7.25	2.80
31	- 70	0.55	21.0	5.18	1.53
32	- 5	0.34	31.9	7.90	2.57
33	-210	1.71	49.0	7.32	2.65
34	-200	2.49	62.0	7.37	3.84
35	-200	5.56	18.1	6.92	2.62
36	-360	2.78	16.7	7.47	2.65
37	-140	2.90	3.3	3.99	3.49
38	-100	1.26	37.3	6.26	2.51
39	- 60	2.05	46.4	7.00	2.60
40	+ 40	<0.01	0.7	1.78	0.04
41	-250	3.21	11.3	9.77	2.46
42	- 60	2.65	50.2	7.10	2.30
43	-120	2.64	34.0	7.76	2.49

Fig. 34.

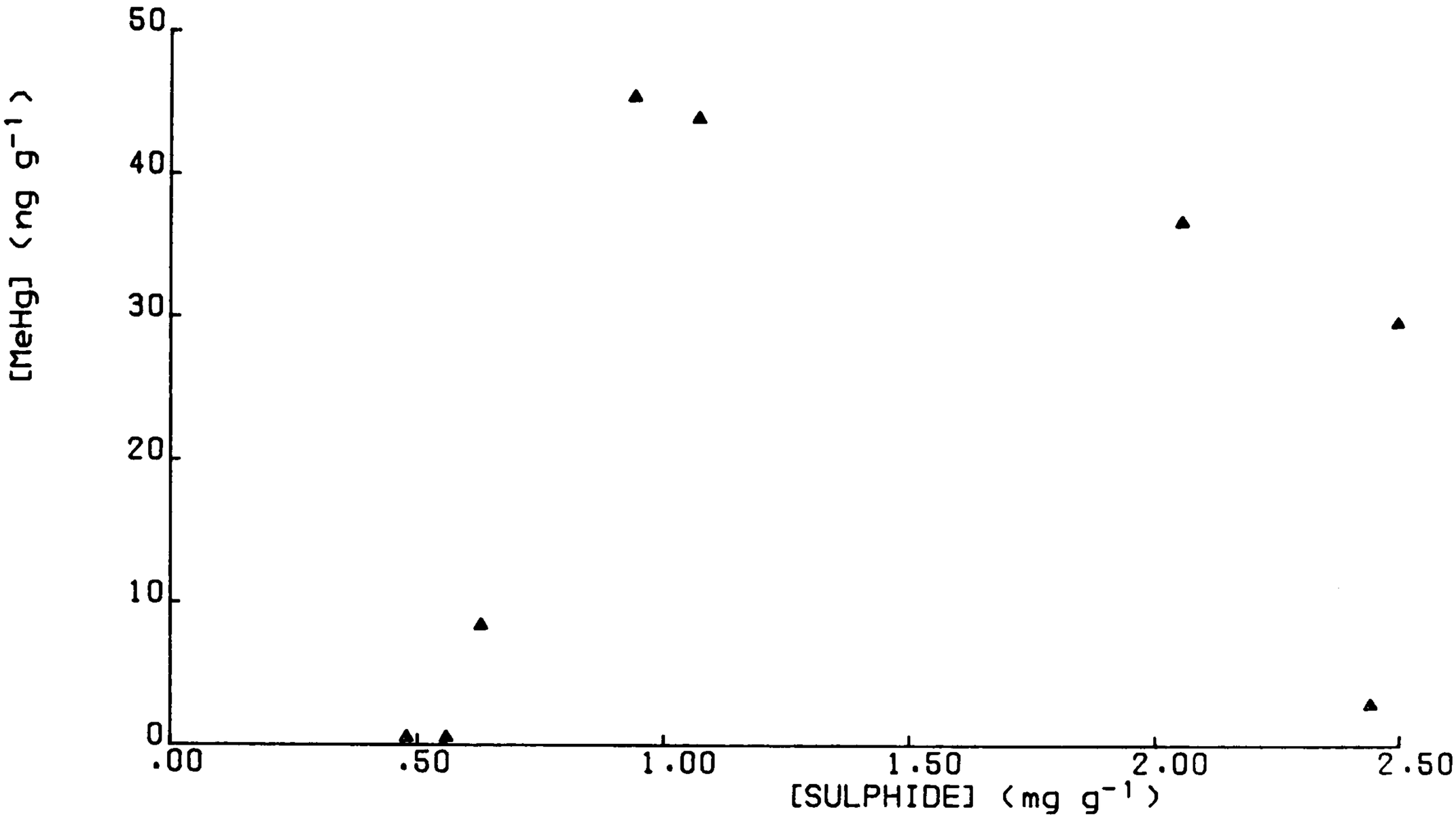
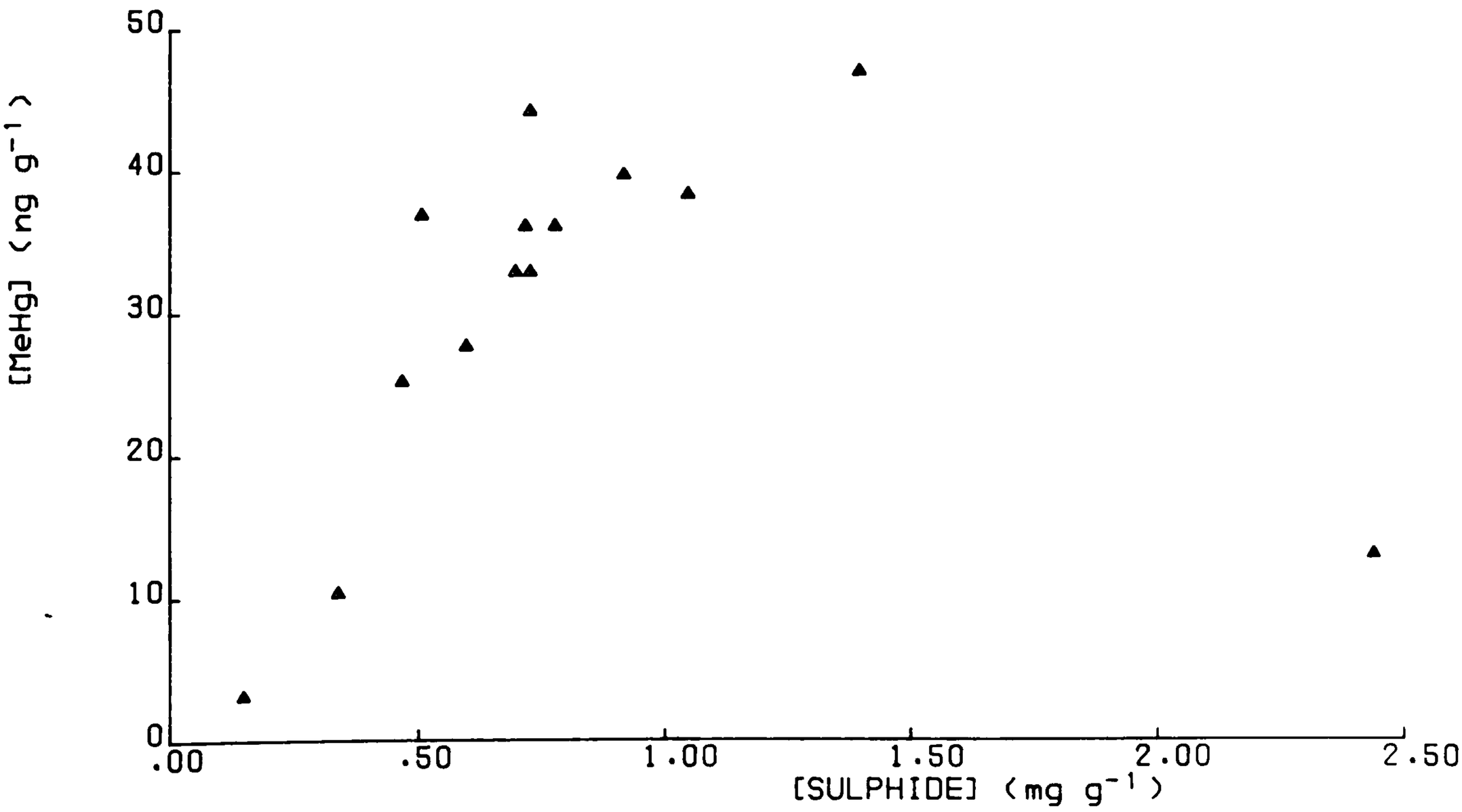

CARRON SURVEY NOVEMBER 1981 : [MeHg] vs [SULPHIDE]
[MeHg] (ng g-1)
50
40
30
20
10
0
.00
.50
1.00
1.50
2.00
2.50
[SULPHIDE] (mg g-1)

Fig. 35.


CARRON SURVEY JULY 1982 : [MeHg] vs [SULPHIDE]
[MeHg] (ng g-1)
50
40
30
20
10
0
.00
.50
1.00
1.50
2.00
2.50
[SULPHIDE] (mg g-1)

correlation between methylmercury and total mercury levels was found also ($r = 0.46$, $P<0.1$).

The results of the November 1982 survey again show a linear relationship between methylmercury levels and low sulphide concentrations (Fig. 36). A least squares analysis of the data points falling below 2.6 mg g^{-1} sulphide gave a linear correlation coefficient of 0.90 ($P<0.001$) and the following equation for the straight line:

$$[MeHg](ng\ g^{-1}) = 21.3[Sulphide](mg\ g^{-1}) + 11.08,$$

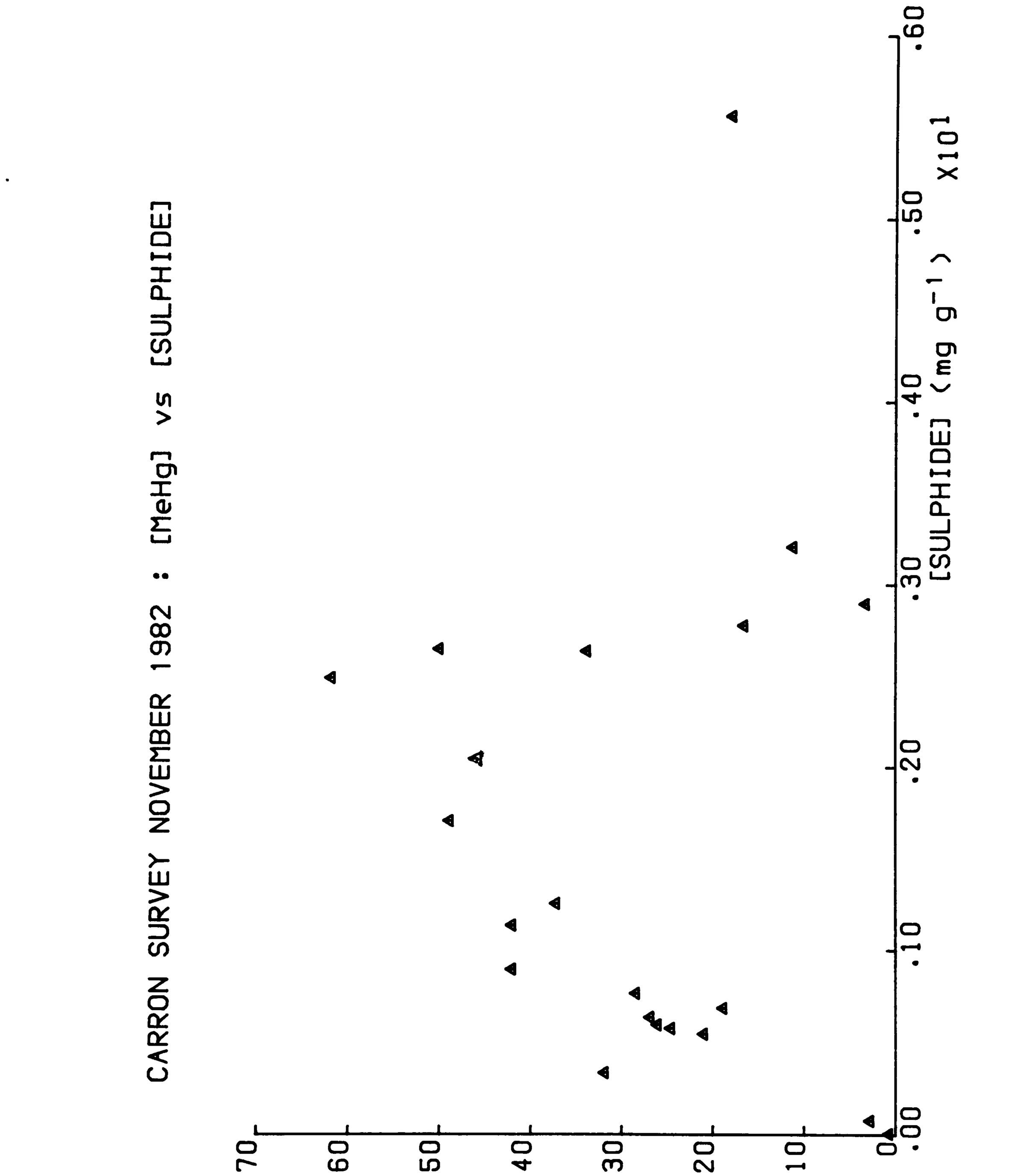
$$S.D. = 7.46$$

A decrease in methylmercury levels at sulphide concentrations greater than 2.6 mg g^{-1} was observed. A poor correlation between methylmercury and total mercury levels also was found ($r = 0.37$, $P<0.1$). Methylmercury and total mercury levels also were found to correlate less well than methylmercury and sulphide levels when the sulphide concentrations were low. The linear correlation coefficient for the methylmercury and total mercury levels of those sediments containing less than 2.6 mg g^{-1} sulphide is 0.60 ($P<0.02$); for methylmercury and sulphide it is 0.90 ($P<0.001$). The organic carbon content (TOC) and Eh values of the sediments collected in the November 1982 survey also were determined. Correlations between TOC, methylmercury and total mercury were investigated along with a correlation between Eh and methylmercury. The results are presented below:-

Relation	r	S.D.
[Hg]TOT. : TOC	0.43 ($P<0.1$)	0.88
[MeHg] : TOC	0.41 ($P<0.1$)	15.69
[MeHg] : Eh	-0.11 ($P>0.1$)	17.08

Better correlations between [MeHg] : TOC and [MeHg] : Eh are found if the data from those sediments containing sulphide

Fig. 36.


CARRON SURVEY NOVEMBER 1982 : [MeHg] vs [SULPHIDE]
[MeHg] (ng g⁻¹)
0.00
10
20
30
40
50
60
70
[SULPHIDE] (mg g⁻¹) X10¹
.00
.10
.20
.30
.40
.50
.60

concentrations greater than 2.6 mg g^{-1} is discarded. The results are presented below:-

Relation	r	S.D.
(1) [MeHg]: TOC	0.58 (P<0.05)	13.98
(2) [MeHg]: TOC	-0.71 (P<0.01)	12.15

The equations for (1) and (2) are as follows:

$$[MeHg]\ (ng\ g^{-1}) = 5.37\ TOC\ (\%) - 2.97$$
$$[MeHg]\ (ng\ g^{-1}) = -0.15\ Eh(mV) + 19.78$$

The composite Figure 37 shows the general relationship found between methylmercury and sulphide levels for this location, the maximum point in the graph occurs between 1.8 - 2.0 mg g^{-1} sulphide.

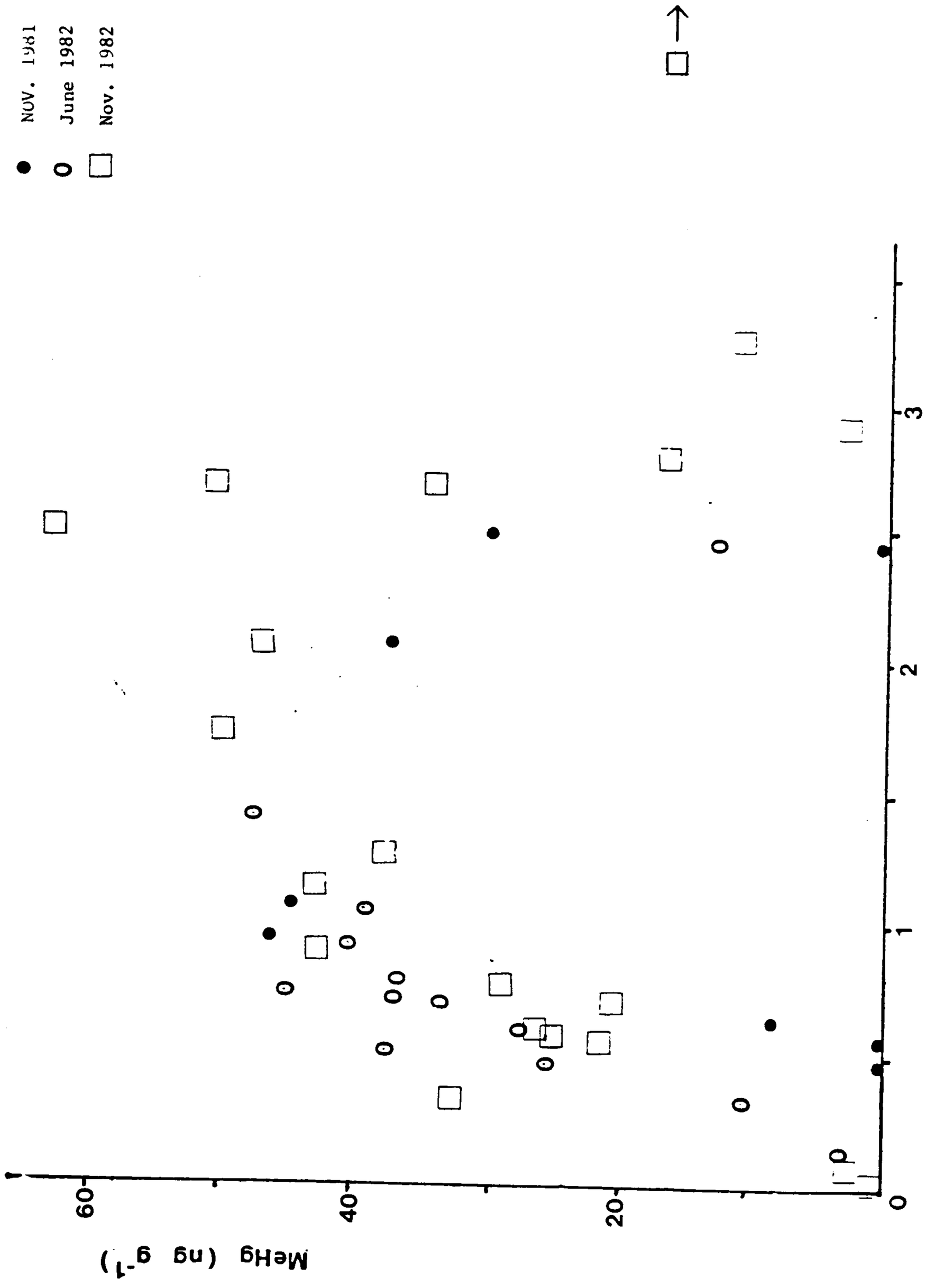
Discussion

The results presented in this thesis demonstrate for the first time methylmercury levels in the Carron.

The results show that methylmercury levels are controlled more by the sulphide content of sediments than by factors such as total mercury levels or organic content. A highly significant linear relationship between methylmercury and sulphide concentrations was found in sediments containing low levels of sulphide; sediments containing high levels of sulphide generally were found to contain low amounts of methylmercury. The importance of sulphide concentration in controlling levels of methylmercury in sediments was confirmed by the results of surveys of other rivers undertaken during the course of this project. The reasons for the observed relationship between the two parameters are discussed later in Chapters 19 and 20.

The maximum points in the [MeHg] vs [Sulphide] graphs for the data of the November 1981 and July and November 1982 surveys occur at slightly different sulphide concentrations; the equations of the straight lines for the linear sections

Fig. 37.


Nov. 1981
June 1982
Nov. 1982
SULPHIDE (mg g-1)
0
1
2
3
MeHg (ng g-1)
0
20
40
60

of the graphs also are different. However, these results were not unexpected as the surveys were undertaken at different times of the year, when sediment microbiological processes proceed at different rates, and not all samples were collected from identical locations in all surveys.

The correlation between total mercury levels and organic carbon contents of Carron sediments is not highly significant. Various workers have investigated mercury/organic carbon relationships and have observed high correlations between mercury and organic contents of sediments[46,47,51,155,156]. However, other workers also have found poor and non-significant correlations between these two parameters (157,158) and selective extraction experiments often do not confirm the association of mercury and organic carbon (159,160). The poor correlation reported here may be due to the limited range of organic carbon found (76% of the samples contained between 4.39% and 8.05% TOC, a range of only 3.66%).

Chapter 14

Mercury in Clyde Estuary Sediments

Description of the Estuary

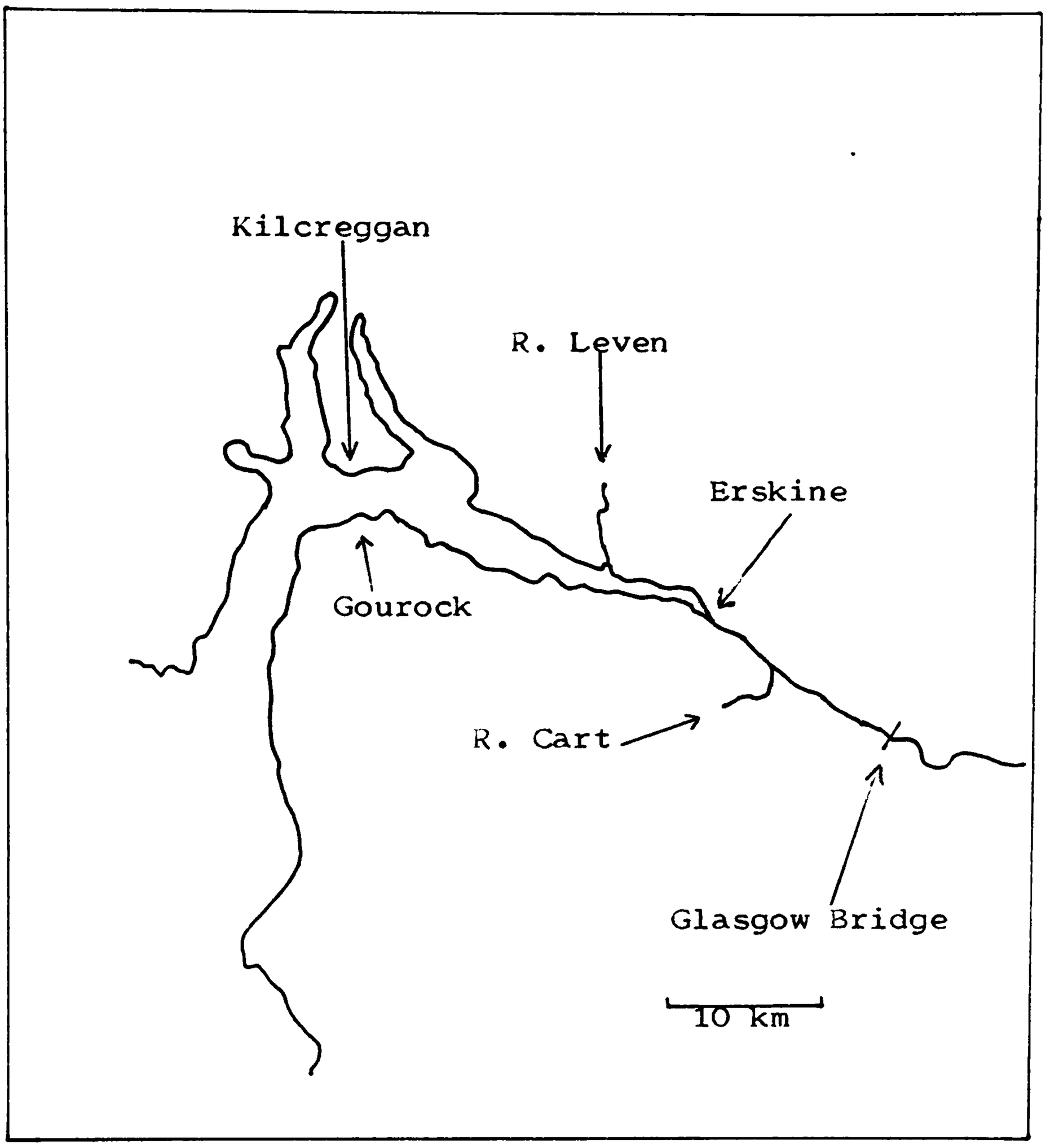
The Firth of Clyde (Fig. 38) can be divided into 2 principal sections. From the seaward limit of the estuary - defined as between Gourock and Kilcreggan - upwards to Erskine, there are large areas of intertidal mudflats. Above Erskine the estuary is narrow and confined largely between man-made banks, with no significant intertidal area. A shipping channel is maintained in the estuary by dredging to a depth of 10 m. It is estimated that the estuary receives 10^5 tons of suspended solids each year, most of which is deposited. Dredging of the shipping channel removes 4×10^4 tons of sediment annually, the remainder of the solids are deposited in areas outside the main channel and on the mudflats. The main tributaries of the estuary are the Rivers Kelvin, Cart, Leven and Clyde, all of which are polluted.

The Firth of Clyde receives pollution inputs from industrial, domestic and agricultural sources. During the summer months, when the flow of water is low, the pollution inputs produce anoxic conditions in the estuary, and vigorous "bubbling" of bio-gas (approximately 70% methane and 30% carbon dioxide) can be seen in some locations. However, there has been an improvement in water quality in recent years, following the clo sure of older sewage works and the installation of new effluent treatment plants in some of the factories discharging into the estuary and its tributaries. The current economic recession also has produced a decline in the amount of effluent discharged to the estuary.

There are no specific industrial inputs of mercury in to

Fig. 38.

Map of the Clyde Estuary



the Clyde. The mercury present in Clyde sediments derives from the widespread use of products containing small amounts of mercury, which eventually is discharged to the environment in urban waste.

The highly anoxic condition of Clyde sediments during the summer months makes this estuary a particularly suitable location for studying mercury -sulphide relationships.

Sampling and Analysis

Two surveys of the Clyde were undertaken in conjunction with the Clyde River Purification Board (C.R.P.B.) during the period June 1982 and June 1983. The section of the estuary from which sediment samples were taken is illustrated in Fig. 38. All samples were collected from bottom sediments; no intertidal sites were available. The samples were collected using a Day grab operated from the C.R.P.B. Marine Survey vessel 'Endrick II'. Eh values were measured on sediments in the grab immediately after collection. Samples were then placed in polythene bottles which were closed with gas-tight caps to prevent exposure of the sediment to air. The samples were frozen using dry ice and transported to the laboratory in Leicester where they were kept in deep freeze until analysis for methylmercury and sulphide content (up to 1 week).

Results

The results of the surveys are presented in Tables 14 and 15.

The data collected for the 1982 survey demonstrates high correlations between the methylmercury content and both the total mercury and sulphide contents of the sediments (Figs. 39 and 40 respectively). A least squares analysis of the data represented in Figs. 39 and 40 gave the following linear correlation coefficients (r) and equations for the straight lines:-

Table 14

Clyde Survey - June 1982

Sample No.	Eh (mV)	[Sulphide] (mg g^{-1})	[MeHg] (ng g^{-1})	[Hg] TOT (ug g^{-1})
0	-177	0.45	5.8	0.76
1	-370	12.59	12.8	1.16
2	-230	3.86	8.8	0.88
3	-100	3.32	5.4	0.77
4	-220	3.12	7.1	0.62
5	-153	0.14	1.5	< 0.05
6	- 40	0.18	2.5	0.50
7	-164	2.23	3.5	0.84
8	-130	3.11	17.0	3.68
9	-166	0.05	3.2	0.05
10	- 40	0.98	6.0	0.33
11	- 70	0.08	< 0.5	< 0.05
12	- 150	0.02	1.0	0.08
13½	-110	0.43	1.9	0.08
15	+190	0.01	< 0.5	< 0.05
17	+214	0.02	< 0.5	< 0.05

Sample Nos. are miles downstream from Glasgow Bridge.

Table 15

Clyde Survey - June 1983

Sample No.	Eh (mV)	Sulphide (mg g^{-1})	MeHg (ng g^{-1})	% Org. C	Hg TOT (ug g^{-1})
0 Anoxic	-350	2.58	15.1	4.59	0.21
0 Oxic	-150	1.23	10.8	12.26	0.28
0 mixed	-240	2.34	14.1	13.28	0.26
½	-200	1.40	9.8	13.24	0.23
1	-180	0.98	1.4	6.33	0.12
1½	-290	4.17	6.1	9.30	0.30
2	-220	1.08	9.9	5.70	0.30
2½	-310	4.02	5.2	7.66	0.37
3	-280	5.86	1.7	6.38	0.50
3½	-230	8.79	4.1	7.57	0.56
4	-230	5.43	5.1	4.80	1.28
5	-230	2.31	10.3	2.65	0.22
6	+ 10	0.30	4.1	1.96	0.04
7	-160	3.52	11.6	3.94	0.28
7½	-130	0.20	1.5	0.62	0.23
8	-210	1.97	14.5	2.24	1.52
8½	-155	0.86	8.8	2.12	0.40
10	+ 85	0.07	< 0.5	0.46	0.21
12	+160	0.04	< 0.5	0.74	< 0.05

Sample Nos. are miles downstream from Glasgow Bridge.

Fig. 39.

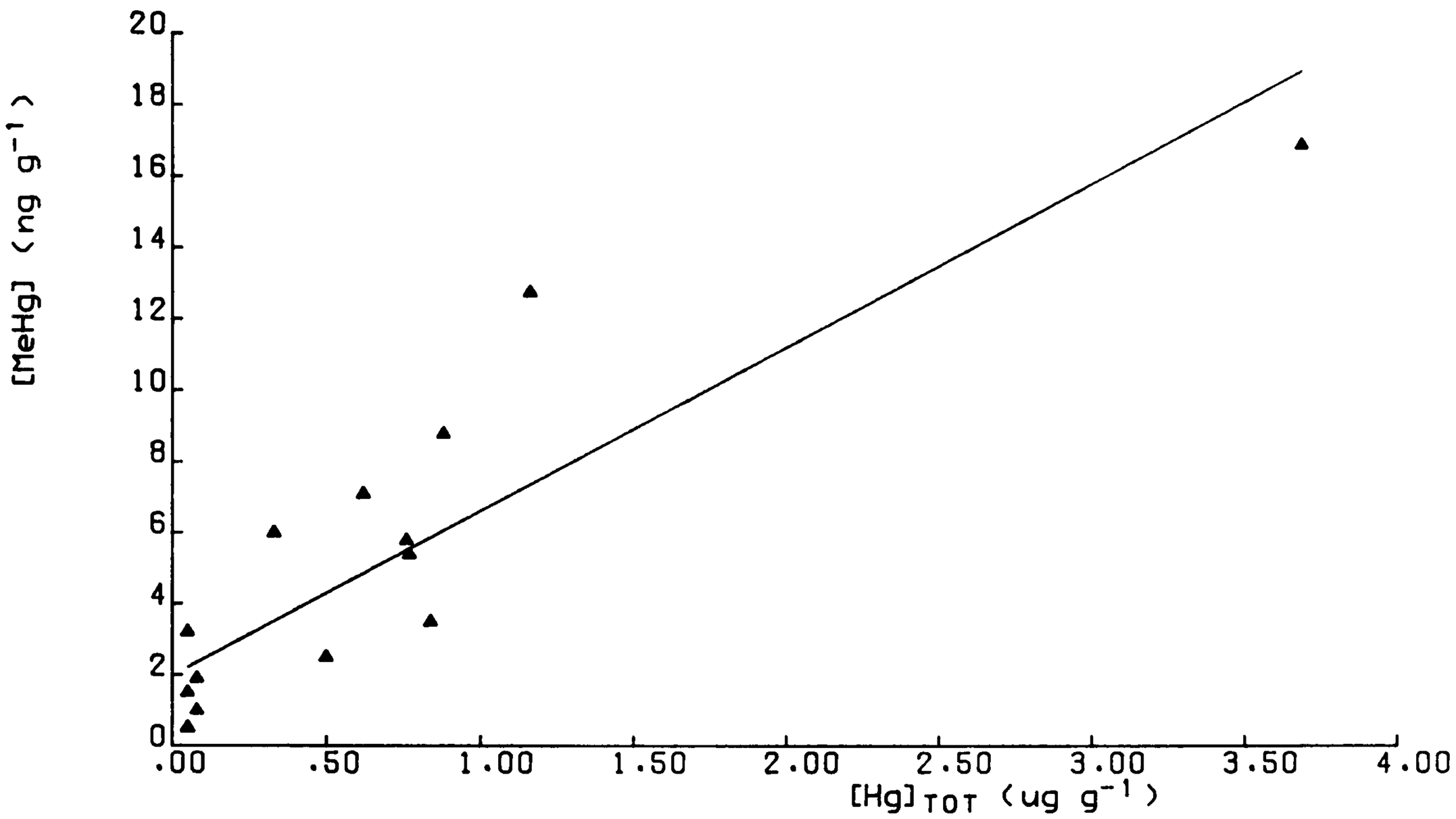

CLYDE SURVEY 1982 : [MeHg] vs [Hg]TOT
[MeHg] (ng g-1)
20
18
16
14
12
10
8
6
4
2
0
.00
.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
[Hg]TOT (ug g-1)

Fig. 40.

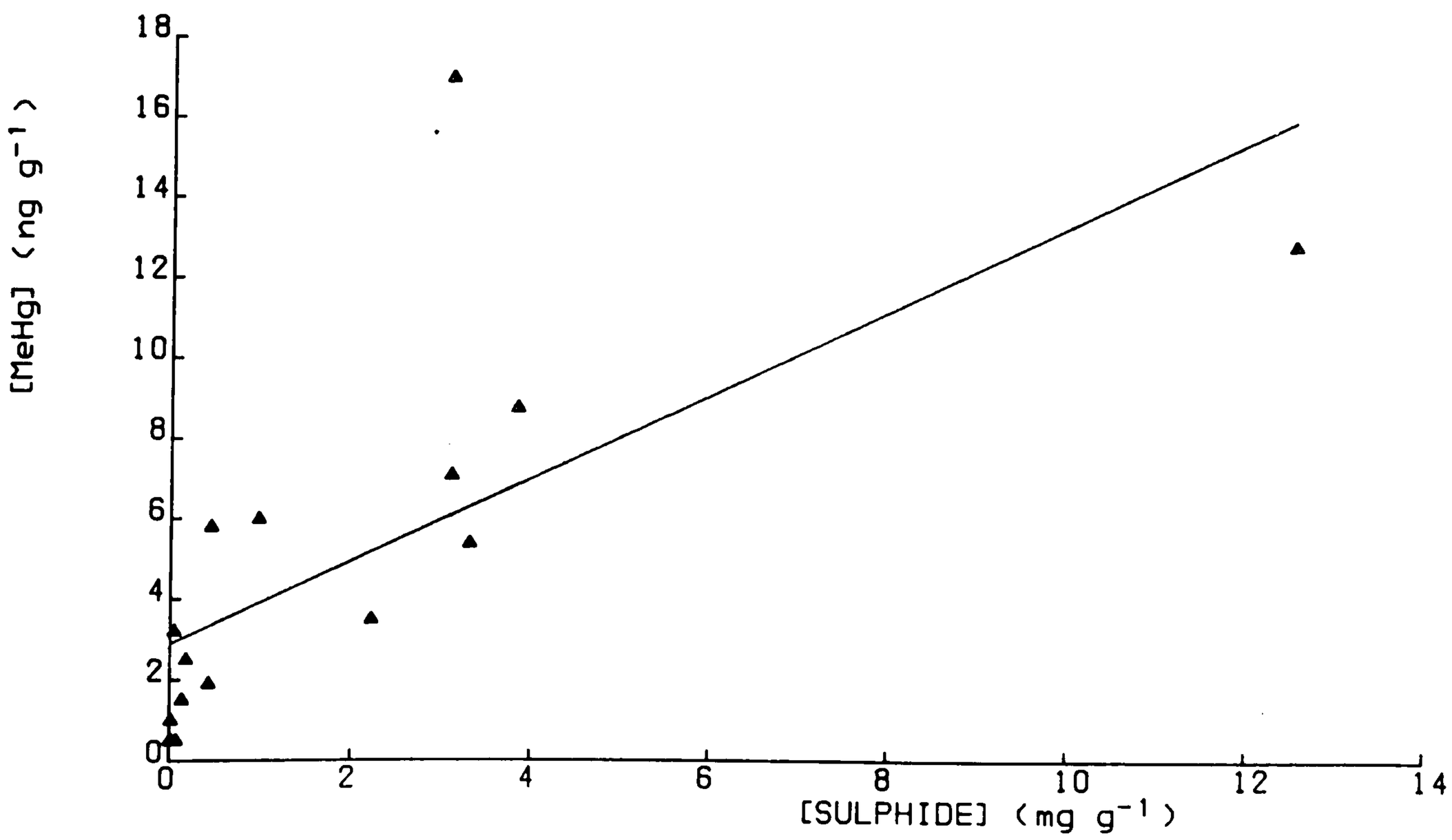

CLYDE SURVEY 1982 : [MeHg] vs [SULPHIDE]
[MeHg] (ng g-1)
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
[SULPHIDE] (mg g-1)

Fig. 39 $[MeHg]\,(ng\ g^{-1}) = 4.65\,[Hg]\,TOT(ug\ g^{-1}) + 1.98$

Standard deviation (S.D.) = 2.30, r = 0.88 ($P<0.001$)

Fig. 40 $[MeHg]\,(ng\ g^{-1}) = 1.04\,[Sulphide]\,(mg\ g^{-1}) + 2.91$

S.D. = 3.52, r = 0.70 ($P<0.01$)

The results of this survey also demonstrate a poor correlation between the methylmercury levels and Eh values of the sediments, r = - 0.55 ($P<0.05$). The negative value obtained for the correlation coefficient implies that methylmercury levels increase as Eh values become more negative.

The results of the 1983 survey demonstrate different relationships between the methylmercury, total mercury and sulphide contents of the sediments. Figs. 41 and 42 show respectively the relationships found between methylmercury and sulphide contents, and, methylmercury and total mercury contents of sediments collected in this survey. It can be seen from Fig. 41 that methylmercury levels rise initially with increase in sulphide concentration, but that after a concentration of about 3 mg g^{-1} of sulphide is exceded, methylmercury levels decay with further increase in sulphide concentrations. A least squares analysis of the low sulphide (<3.00 mg g^{-1}) data of Fig. 41 demonstrated a good linear relationship between methylmercury and sulphide levels in the sediments; the linear correlation coefficient and equation for the straight line are as follows:

$$[MeHg]\,(ng\ g^{-1}) = 5.46\,[Sulphide]\,(mg\ g^{-1}) + 1.34$$

S.D. = 2.61, r = 0.89 ($P<0.001$)

Fig. 42 indicates the lack of any kind of relationship between methylmercury and total mercury levels; a least squares analysis of the data produced a linear correlation coefficient of 0.20 ($P>0.1$)

Correlations between methylmercury levels, Eh values and

Fig. 41.

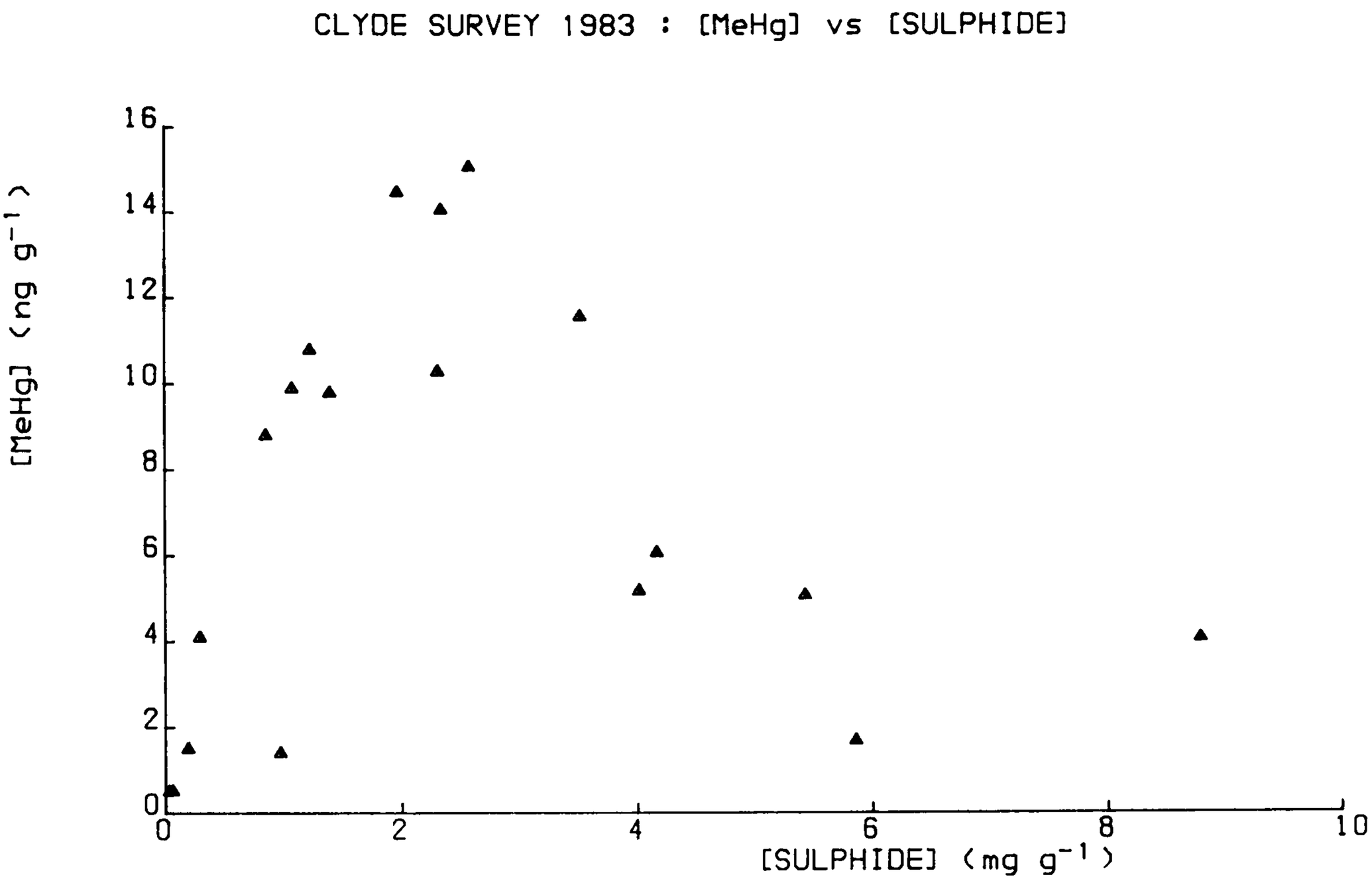
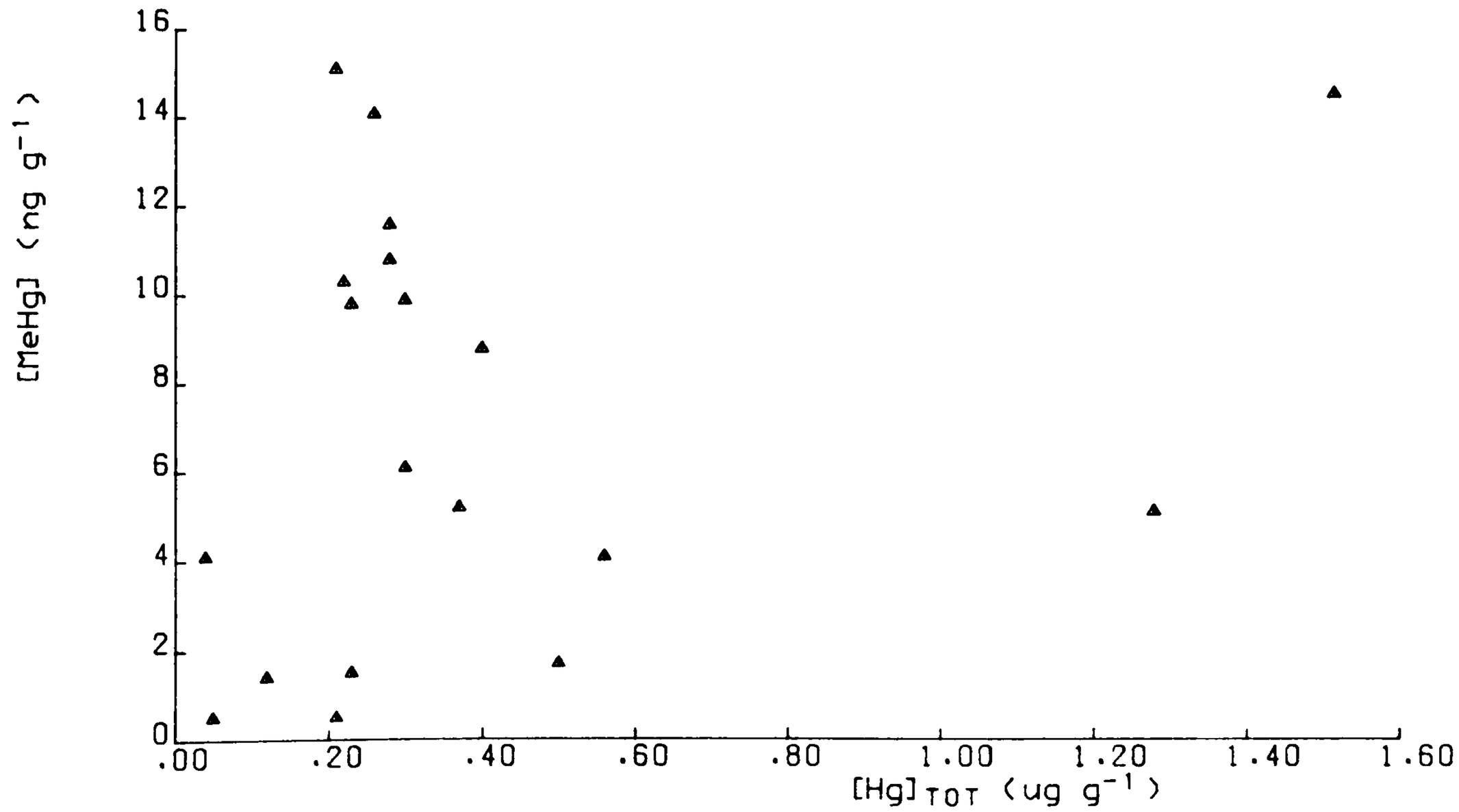

CLYDE SURVEY 1983 : [MeHg] vs [SULPHIDE]
[MeHg] (ng g-1)
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
[SULPHIDE] (mg g-1)

Fig. 42.


CLYDE SURVEY 1983 : [MeHg] vs [Hg]TOT
[MeHg] (ng g-1)
16
14
12
10
8
6
4
2
0
.00
.20
.40
.60
.80
1.00
1.20
1.40
1.60
[Hg]TOT (ug g-1)

organic carbon contents of sediments collected in this survey also were investigated, along with the relationship between total mercury levels and organic carbon contents of the sediments. The results are presented below:-

Relation	r	S.D.
[MeHg] : Eh	-0.50(P<0.05)	4.39
[MeHg] : TOC	0.35(P>0.1)	4.75
[Hg]TOT : TOC	-0.07(P>0.1)	0.39

Better correlations between [MeHg] : Eh and [MeHg] : TOC are found if the data from those sediments containing sulphide concentrations greater than 3.0 mg g^{-1} is discarded. The results are presented below:-

Relation	r	S.D.
(1) [MeHg] : Eh	-0.79(P<0.01)	3.55
(2) [MeHg] : TOC	0.49(P<0.1)	5.00

The equations for (1) and (2) are as follows:-

$$[MeHg]\,(ng\ g^{-1}) = -0.03\ Eh\ (mV) + 3.55$$

$$[MeHg]\,(ng\ g^{-1}) = 0.56\ TOC\ (\%) + 4.96$$

Discussion

The results of both surveys demonstrate the importance of sulphide concentration as a factor in controlling methylmercury levels in the sediment environment. However, different relationships between the methylmercury and sulphide content of sediments were found for the 2 surveys. The results of the 1983 survey demonstrate a maximum point in the methylmercury/sulphide relationship and bear a close resemblance to results obtained from surveys of the River Carron (Chapter 13). The results of the 1982 survey fail to demonstrate a maximum point in this relationship and are similar to the results obtained from surveys of

estuaries in S.W. England (Chapter 12) where sulphide concentrations tend to be low - <2.00 mg g^{-1} . It is worth noting, however, that only 3 samples collected in the 1982 survey contained more than 3.11 mg g^{-1} of sulphide, this being the sulphide concentration associated with the highest level of methylmercury and thus perhaps the concentration at which a maximum point in the [MeHg] vs [Sulphide] graph may have occurred. One of the 3 samples contained an unusually high sulphide concentration of 12.15 mg g^{-1}. This was the highest level of sulphide found in any sample collected during the course of this work, and may have been due to the recent input into the sampling area of a pollutant high in sulphide content. If such an input had occurred at a time in proximity to sampling, then the methylmercury content of the sediments may not have reached an equilibrium value, and this is a possible explanation for the unexpectedly high methylmercury level found in this sample.

The results of the 1982 survey also are unusual in that a high correlation between methylmercury and total mercury levels are found. Other surveys undertaken during the course of this project failed to produce significant correlations between methylmercury and total mercury levels in sediments, the exceptions being the 1982 Plym and 1983 Mersey surveys. The high correlation reported here may be a result of the wide range of sediment types sampled; these ranged from relatively clean sands to muds containing various amounts of pollutants. This point is discussed further in Chapter 15 when results from surveys of the Mersey estuary are considered. For the 1983 survey, a greater number of samples were collected from a smaller section of the estuary. Many of these samples were found to contain similar levels of total mercury, and the methylmercury contents of the sediments were found to be determined more by sulphide concentration than total mercury concentration. The reasons for the observed relationship

between the two parameters are discussed in Chapters 19 and 20.

Finally, the total mercury and organic carbon contents of the Clyde sediments were found to be unrelated. As was noted in Chapter 13, other workers have also found insignificant correlations between these 2 parameters in the sediment environment.

Chapter 15

Mercury in Mersey Estuary Sediments

Description of the Estuary

The Mersey estuary (Fig. 43) can be divided into three sections:-

(1) Howley Weir to the Runcorn-Widnes gap. This section of the estuary is relatively narrow, and below Warrington it runs largely through agricultural land.

(2) Runcorn Gap to Dingle Point. This section of the estuary consists of extensive mudflats, and is bounded on the south side by the Manchester Ship Canal. The area is heavily industrialised.

(3) Dingle Point to the Rock Lighthouse. This section of the estuary is known as the 'Narrows'. In addition to being small in width, the estuary is also relatively straight and deep at this point. The whole section is bounded by embankments and dock systems.

The two principal freshwater inflows to the estuary are:-

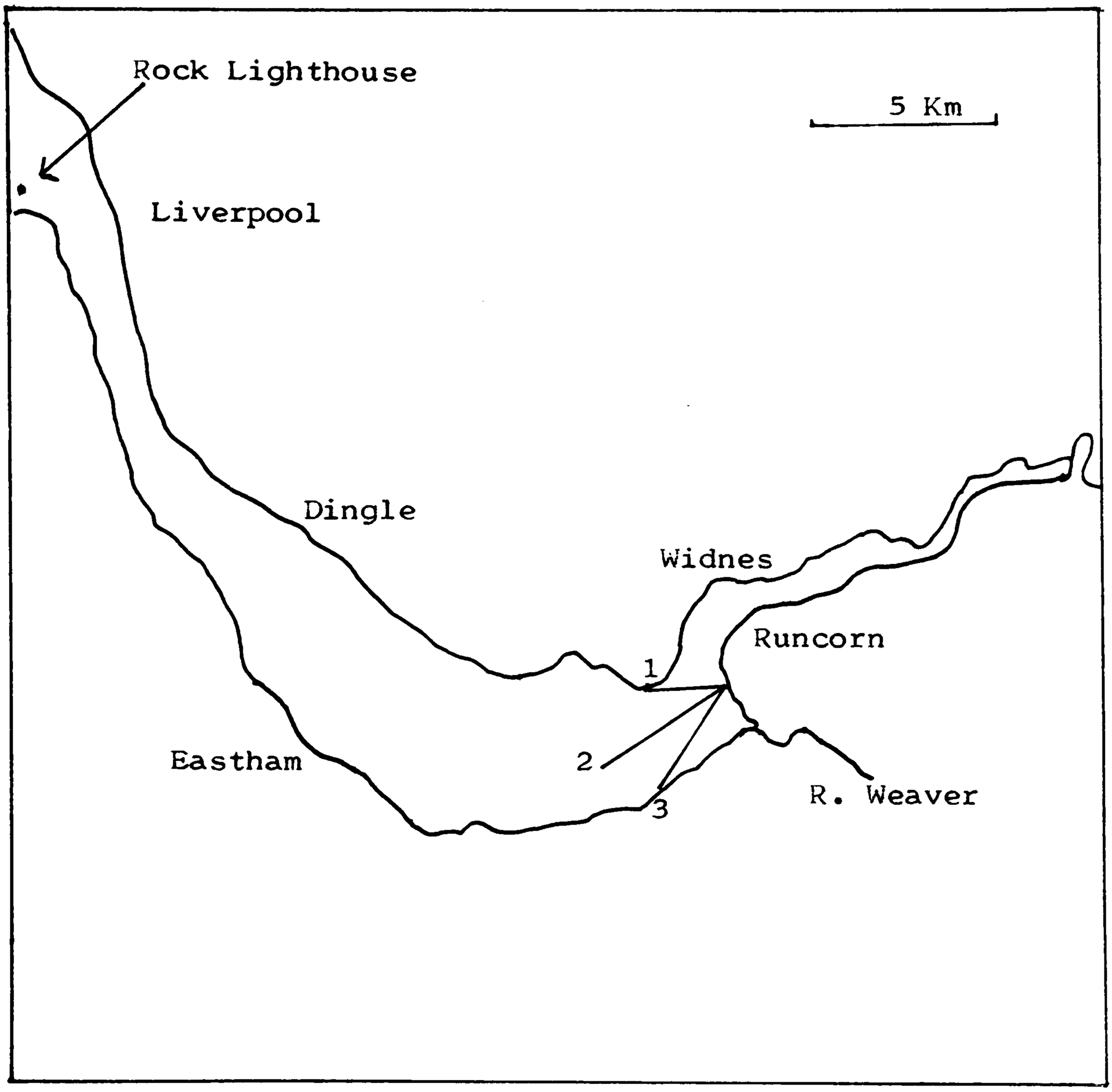
(1) The River Mersey itself, flowing over Howley Weir.

(2) The Manchester Ship Canal, which receives the River Weaver, entering the estuary at Eastham Lock. At times of high flow in the Weaver, excess water is sluiced out of the Ship Canal into the Mersey some 18 Km upstream of Eastham.

These two inflows, together with other minor freshwater inputs, carry large amounts of domestic and industrial wastes. There are also numerous discharges of crude sewage direct to the estuary.

Fig. 43.

Map of the Mersey Estuary



Key to transects : -

1 : T 15

2 : T 15 a

3 : T 15 b

Fine material (silt and clay particles) is carried into the estuary by the freshwater flow, and when run-off is high, these amounts can be appreciable[161]. The organic material in the freshwater tends to combine with the silt and the fine particulates flocculate and settle out at the freshwater/saltwater interface, the point of settlement varying with the tidal state. The settled sediment is carried upstream in the bottom flow and tends to accumulate in the upper estuary. In addition to inputs of sediments from freshwater sources, there is evidence[162,163] to show that inshore movement of bottom water in Liverpool Bay also results in sediments being carried into the estuary. Price and Kendrick[164] have estimated that between 1861 and 1955, 8.5×10^7 cubic meters of material had accumulated in the upper estuary. Channels are maintained through the Mersey by dredging, and approximately 7×10^6 tons of material are removed from the estuary each year. The areas dredged are Eastham Channel, Garston Channel, Docks and Dock entrances, New Brighton Shoal and the sea channels. The dredged material is dumped in Liverpool Bay.

The Mersey estuary is considered to be heavily polluted. the principal sources of pollution are sewage, normally untreated, discharged by the local authorities adjacent to the river, and trade effluent, discharged by manufacturers. The largest pollution loads originate from the Liverpool conurbation and from trade effluent at Stanlow. There is also a point source of mercury input from chloralkali plants at Runcorn.

Sampling and Analysis

The work on the Mersey estuary described in this chapter is, in part, an extension of the work done by Morton[102] and Bartlett[103]. Both of these workers had analysed Mersey sediments for methylmercury and total mercury concentrations and had found significant correlations between

the two parameters ($P<0.05$ in the case of Morton, and $P<0.001$ in Bartlett's work). Bartlett, in addition, had determined the organic carbon and silt content of Mersey sediments and had found high correlations ($P<0.001$) between the following parameters: [Hg]TOT : TOC, [Hg]TOT : silt and [MeHg] : silt. However, the relationship between in situ methylmercury levels and the degree of anoxicity of the sediments had not been investigated. It was, therefore, decided to undertake another survey of the estuary, principally to ascertain whether the methylmercury and sulphide levels in the sediments were related in a manner similar to that observed in sediments of other rivers. Morton and Bartlett's work had shown that the highest concentrations of mercury were present in sediments along the south bank of the estuary, between Weston and Frodsham; this section of the estuary was therefore chosen for survey.

During August 1983, a survey of the estuary was undertaken in conjunction with the North West Water Authority. Sediment samples were collected at low water from intertidal sites using a small hovercraft. The sampling stations were points along the transects illustrated in Fig. 43. The samples were frozen with dry ice soon after collection, and kept in deep freeze until they could be analysed for methylmercury and sulphide content (up to 1 week).

Results

The results of the survey are presented in Table 16.

Mersey sediments were found to contain lower sulphide levels than those found generally in sediments of other polluted rivers, e.g. the Carron and Clyde. Only one sediment was found to contain a sufficiently high sulphide concentration to present evidence of a maximum point in the methylmercury/sulphide relationship (Fig. 44). The other data points in Fig. 44 indicate that methylmercury

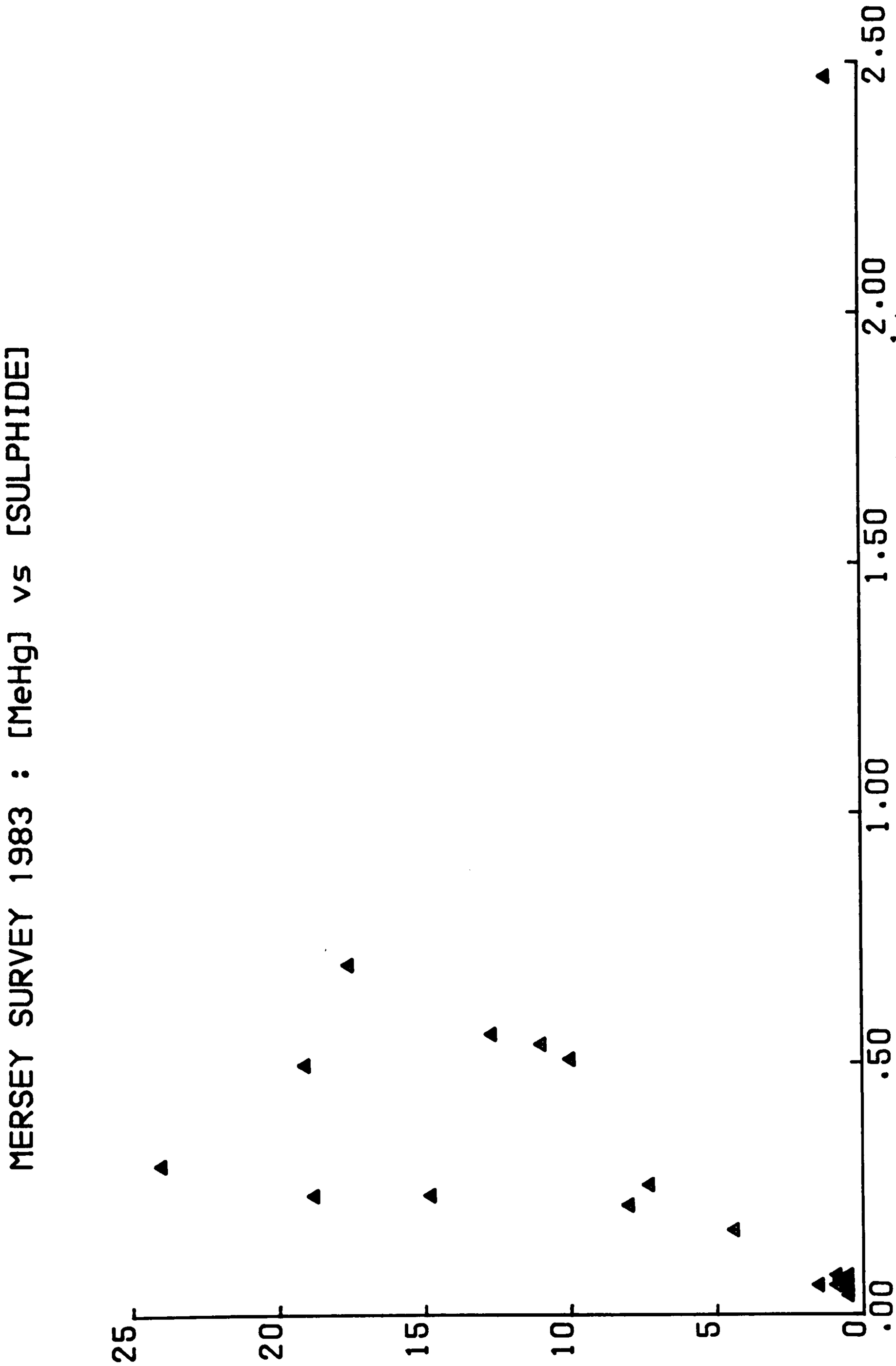
Table 16

Mersey Survey - August 1983

Sample No.	Eh (mV)	[Sulphide] (mg g^{-1})	[MeHg] (ng g^{-1})	TOC (%)	[Hg] TOT (ug g^{-1}
15 1	-100	0.08	0.9	1.10	0.08
15 2	-200	0.50	19.1	2.41	1.85
15 3	-180	0.54	11.0	2.81	1.49
15 4	-150	0.24	14.8	3.07	1.59
15 6	- 50	0.06	0.5	1.18	0.16
15 7	- 50	0.04	0.5	1.25	< 0.05
15a1	- 20	0.08	0.5	1.61	0.05
15a2	0	0.07	0.8	0.84	< 0.05
15a3	0	0.05	< 0.5	0.97	< 0.05
15a4	+100	0.06	< 0.5	0.92	0.08
15a5	+100	0.06	0.9	0.85	0.08
15a6	+ 80	0.06	1.51	0.87	0.05
15b1	-180	0.56	12.7	1.85	1.20
15b2	-150	0.30	24.0	5.70	3.81
15b3	-150	0.70	17.6	3.89	2.84
15b4	-120	0.26	7.3	2.07	2.38
15b5	-190	0.51	10.0	3.92	2.88
15b6	-250	2.47	1.1	1.71	3.30
15b7	-150	0.24	18.8	4.52	3.03
15b8	-160	0.22	8.0	5.01	3.21
15b9	-150	0.17	4.4	5.06	4.01

The sampling stations are equidistant along the transects and are numbered outwards from the bank (see Fig. 43).

Fig. 44


MERSEY SURVEY 1983 : [MeHg] vs [SULPHIDE]
[MeHg] (ng g⁻¹)
[SULPHIDE] (mg g⁻¹)
0.00
.50
1.00
1.50
2.00
2.50
0
5
10
15
20
25

and sulphide levels in Mersey sediments are linearly related; this is borne out by a least squares analysis of the data, from which the following equation and correlation coefficient are obtained:-

$$[MeHg]\,(ng\ g^{-1}) = 27.48\,[Sulphide]\,(mg\ g^{-1}) + 1.12$$

$$S.D. = 5.39, \qquad r = 0.74\ (P<0.001)$$

Figs. 45 - 48 show, respectively, plots of [MeHg] vs [Hg]TOT, [Hg] TOT vs TOC, [MeHg] vs TOC and [MeHg] vs Eh. From a least squares analysis of the data presented in Figs. 45 - 48, the following equations and correlation coefficients are obtained:-

$$[MeHg]\,(ng\ g^{-1}) = 3.33\,[Hg]\,TOT(ug\ g^{-1}) + 2.29$$

$$S.D. = 6.15, \qquad r = 0.63\ (P<0.01)$$

$$[Hg]\,TOT(ug\ g^{-1}) = 0.81TOC(\%) + 0.45$$

$$S.D. = 0.70, \qquad r = 0.89\ (P<0.001)$$

$$[MeHg]\,(ng\ g^{-1}) = 3.46TOC(\%) - 1.12$$

$$S.D. = 5.51, \qquad r = 0.72\ (P<0.001)$$

$$[MeHg]\,(ng\ g^{-1}) = -0.04Eh(mV) + 3.14$$

$$S.D. = 6.32, \qquad r = -0.60\ (P<0.01)$$

Better correlations between [MeHg] and [Hg]TOT, and, [MeHg] and Eh, are found if the high sulphide data point is excluded from the statistical analysis:-

$$[MeHg]\,(ng\ g^{-1}) = 3.90\,[Hg]\,TOT(ug\ g^{-1}) + 2.07$$

$$S.D. = 5.53, \qquad r = 0.72\ (P<0.001)$$

$$[MeHg]\,(ng\ g^{-1}) = -0.06Eh(mV) + 2.80$$

$$S.D. = 5.51, \qquad r = -0.73\ (P<0.001)$$

Fig. 45.

MERSEY SURVEY 1983 : [MeHg] vs $[Hg]_{TOT}$

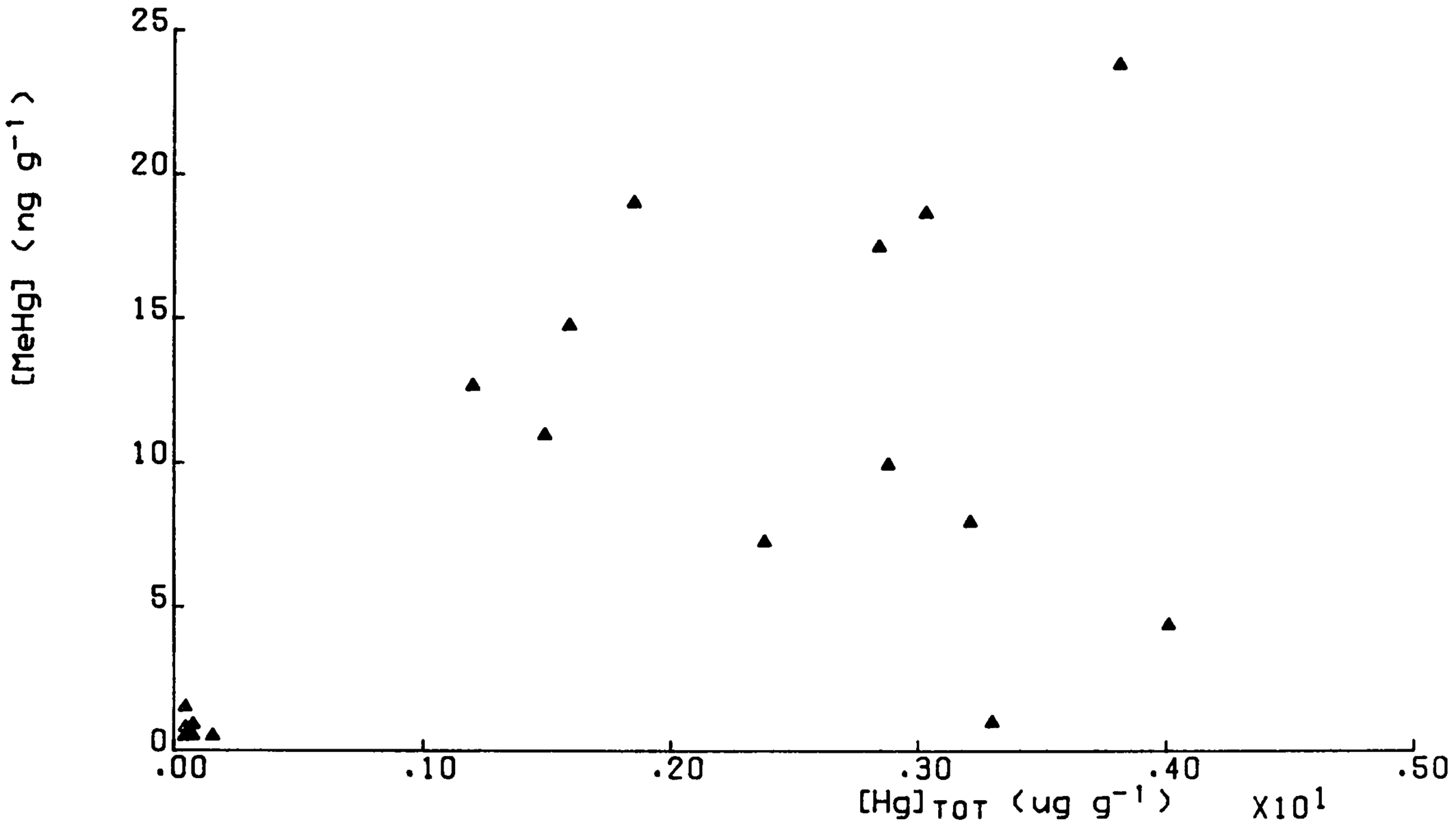


Fig. 46.

MERSEY SURVEY 1983 : $[Hg]_{TOT}$ vs TOC

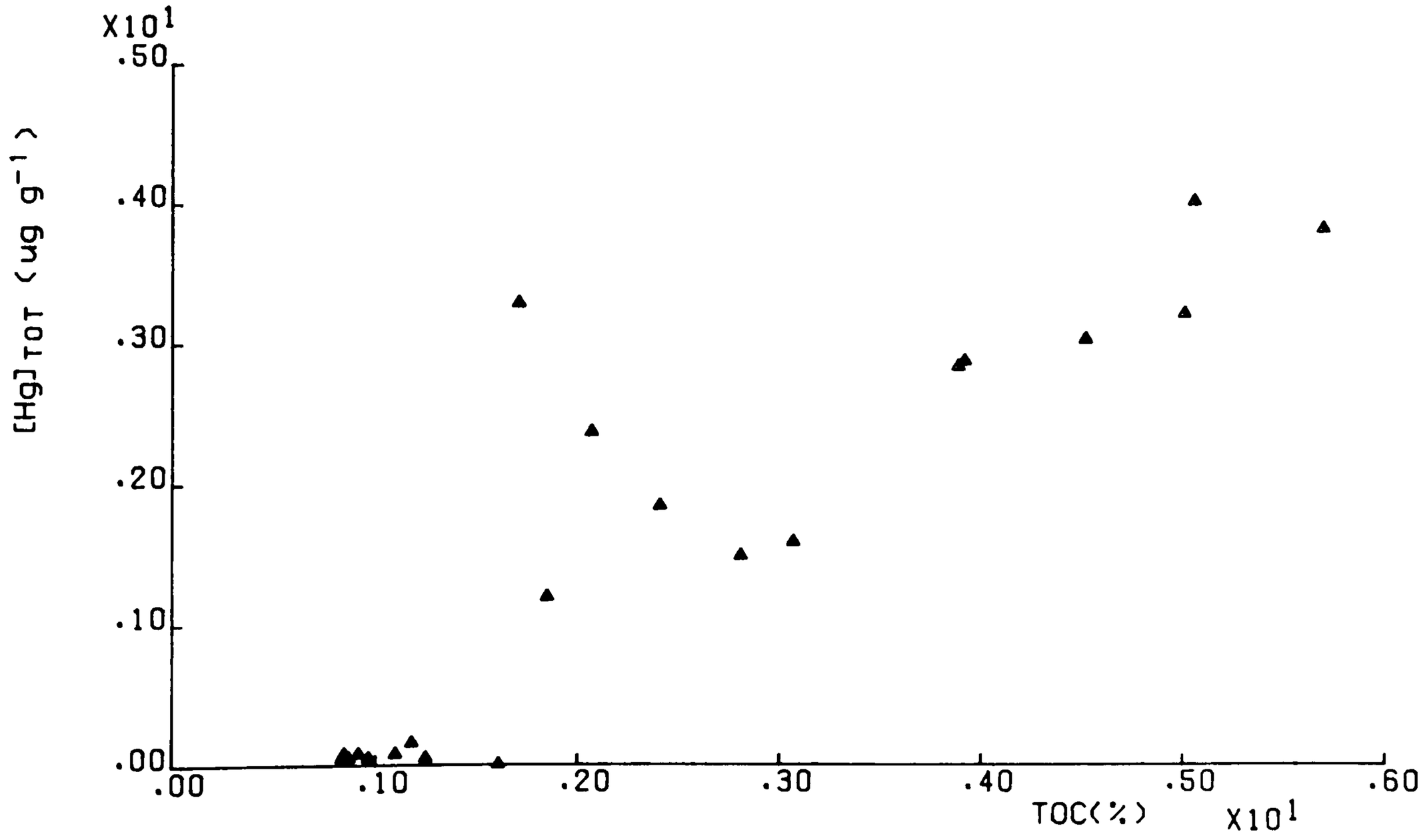


Fig. 47.

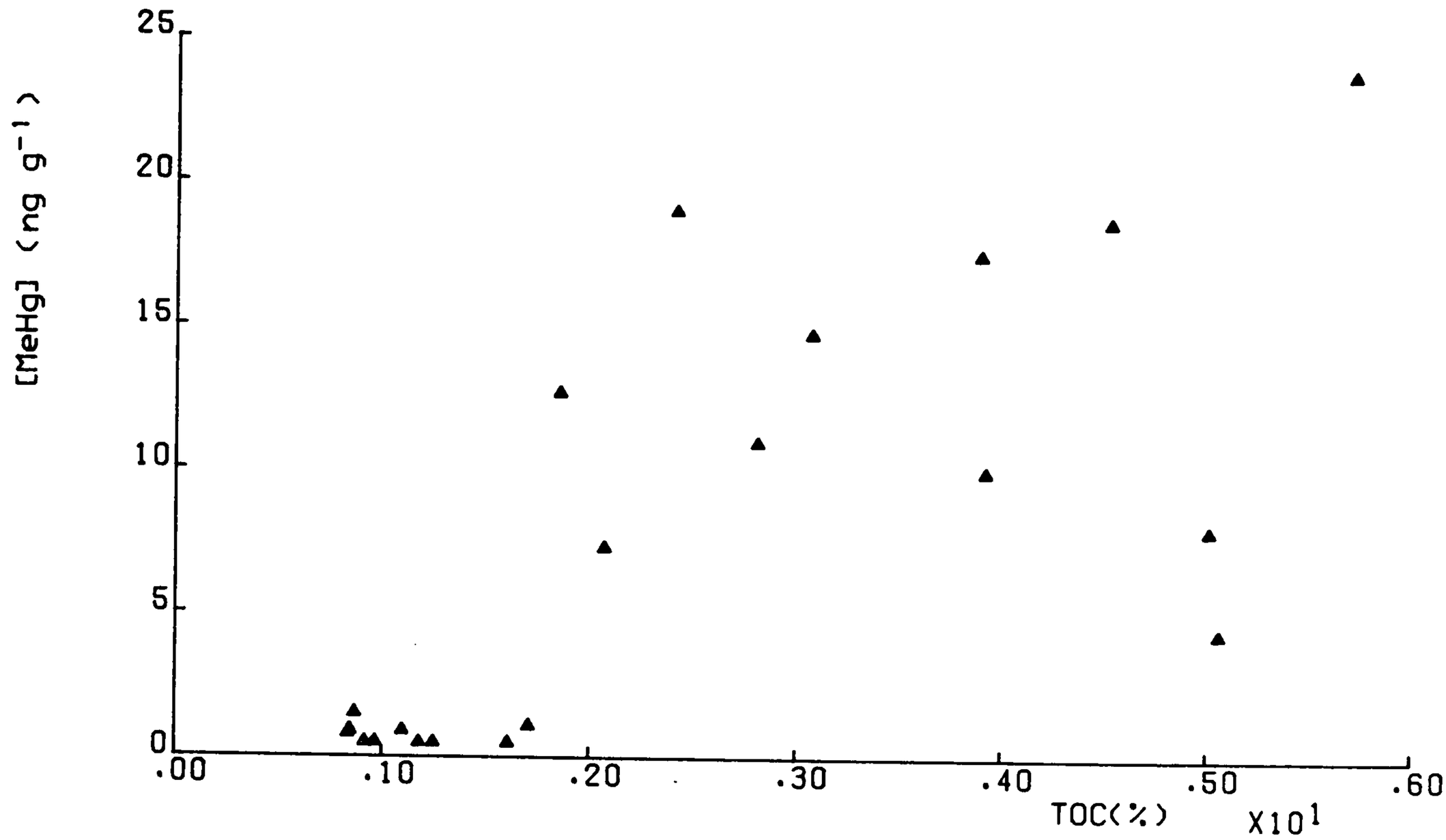
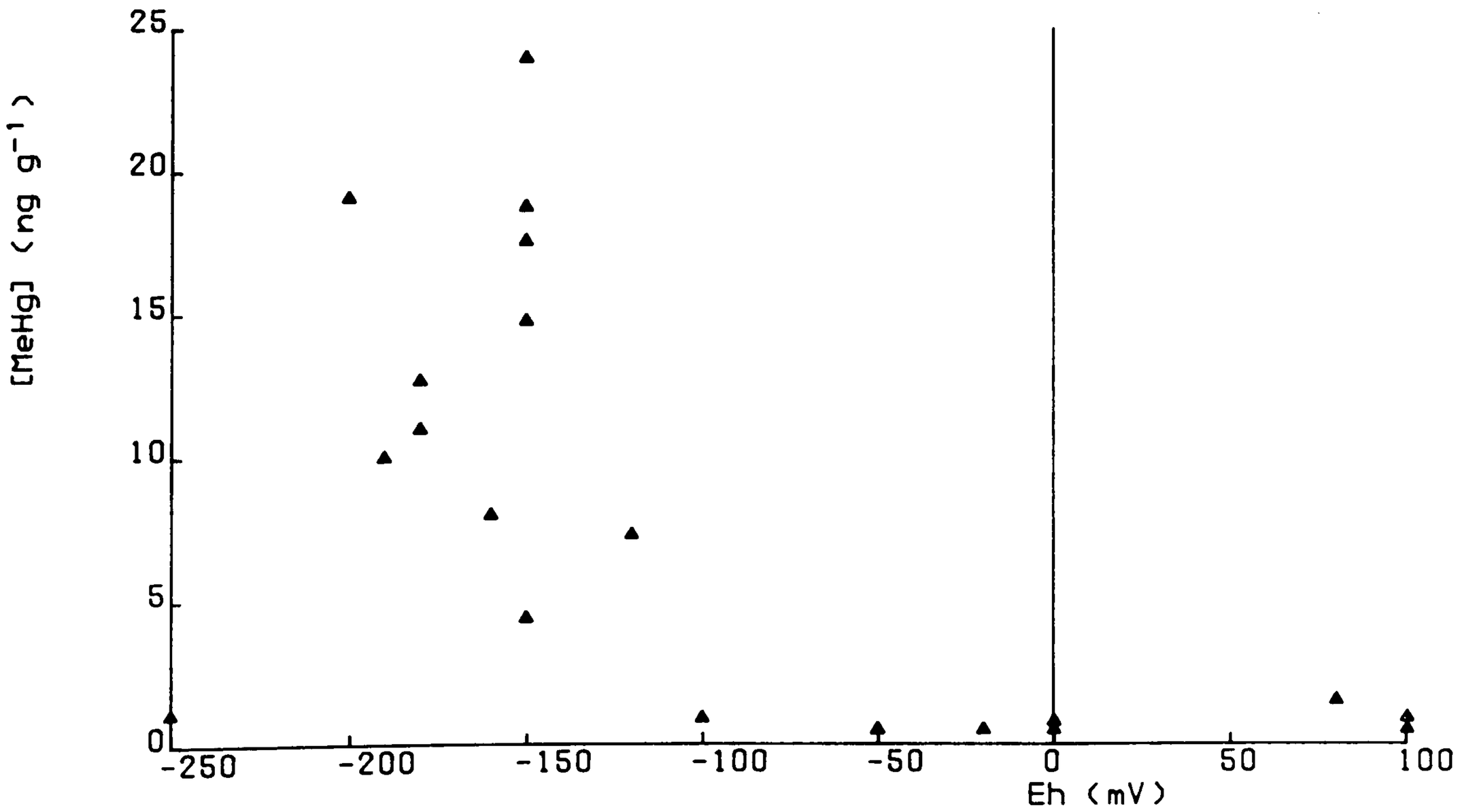

MERSEY SURVEY 1983 : [MeHg] vs TOC
[MeHg] (ng g-1)
25
20
15
10
5
0
.00
.10
.20
.30
.40
.50
.60
TOC(%)
X10 1

Fig. 48.


MERSEY SURVEY 1983 : [MeHg] vs Eh
[MeHg] (ng g-1)
25
20
15
10
5
0
-250
-200
-150
-100
-50
0
50
100
Eh (mV)

Discussion

The results of this survey demonstrate, once again, a connection between methylmercury and sulphide levels in the sediment environment. The relationship between these two parameters is discussed fully in Chapters 19 and 20.

The results also demonstrate the importance of total mercury levels in sediments in determining methylmercury concentrations. The results of other surveys undertaken during the course of this project often have failed to demonstrate any relationship between sediment methylmercury and total mercury levels. The high correlation between the two parameters reported here seems to be a consequence of both the low sulphide contents of the sediments (only one sediment was found to contain a sufficiently high sulphide content to depress the methylmercury level), and the range and proportion of sediment types sampled. Approximately half of the samples were relatively clean sands, and half were muds containing greater amounts of pollutants; thus a large range in total mercury levels was determined. The results of those surveys in which samples were taken predominantly from sediments containing a narrow range of total mercury levels, e.g. the Carron and second Clyde surveys, have shown that sulphide concentration, and not total mercury concentration, is the more important factor in controlling methylmercury levels.

The results of the Mersey survey also demonstrate the affinity of mercury for organic matter, a result which has been reported by other workers[46,47,51]. Again, the results of other surveys undertaken during the course of the project have more often than not failed to show a connection between the total mercury and organic carbon contents of sediments. The discrepancy arises as mercury concentrations in sediments of the majority of the estuaries surveyed are governed more by local pollution inputs than by chemical parameters within the sediments.

Finally, a comparison can be made with some of the data from Bartlett's 1978 survey of the Mersey. Seven sampling stations were common to each survey; the results are compared below:-

Total Mercury Levels (ug g^{-1})

Sampling Station	1978	1983	Difference
15a1	2.65	0.05	-2.60
15a6	0.31	0.05	-0.26
15b1	3.37	1.20	-2.17
15b2	4.15	3.81	-0.34
15b4	2.72	2.38	-0.34
15b8	3.77	3.21	-0.56
15 3	0.18	1.49	+1.31
Average =	2.45	1.74	-0.71

Methyl Mercury Levels (ng g^{-1})

Sampling Station	1978	1983	Difference
15a1	3.2	0.5	-2.7
15a6	4.8	1.5	-3.3
15b1	20.1	12.7	-7.4
15b2	43.3	24.0	-19.3
15b4	16.0	7.3	-8.7
15b8	13.1	8.0	-5.1
15 3	1.5	11.0	+9.5
Average =	14.6	9·3	-5·3

Although total mercury and methylmercury levels at six of the seven sampling stations are seen to have decreased between 1978 and 1983, a matched pairs statistical analysis of the data (see Chapter 11) shows that the decrease in total mercury and methylmercury levels is not significant. However, if the data obtained from sampling station 15 3 is discarded, t values of 2.43 and 3.11 are obtained for the differences between the levels of total mercury and

methylmercury respectively. For the t- distribution with 5 degrees of freedom, the 5 % critical value of |t| is 2.57. Thus, it can be concluded that whilst the difference in methylmercury levels is probably significant, the test statistic provides insufficient evidence (though only marginally) of a difference in total mercury levels between the results of the 1978 and 1983 surveys. The average values of the methylmercury : total mercury percentage ratio also may be compared. The values are 0.60 and 0.54 for the 1978 and 1983 surveys respectively. The extremely close proximity of these two values points to a consistent methylmercury/ total mercury relationship in Mersey sediments.

SECTION 4

SYNTHETIC WORK

Chapter 16

Differential Incubation Experiments

Mercury in natural waters is strongly associated with suspended particulate matter. This has been demonstrated by the results of both filtration[165,166,167] and centrifugation experiments[168,169], which have shown that particulate forms of mercury (of pore size greater than 0.45 um in the case of filtration experiments) may account for well over 50 per cent of the total mercury present in river, estuarine and sea water. Even in glacial meltwaters almost quantitative association of mercury with particulates has been found[165]. The occurrence of mercury in particulate form can be attributed to two main causes:

(1) precipitation of an insoluble form of mercury, and

(2) adsorption or coprecipitation of mercury on or with solid particles both of an organic or inorganic nature.

Hem[170] calculated the stability of a number of sparingly soluble mercury compounds, and concluded that only elemental mercury and mercuric sulphide are stable forms in typical natural water conditions. Morel et al[171] calculated that mercuric sulphide might be the prevalent suspended form of mercury in fresh waters under reducing conditions and in sewage effluents, and Jenne[172] and Jonasson and Timperley[173] have pointed to possible precipitation of mercuric sulphide as a cause of mercury accumulation in bed sediments.

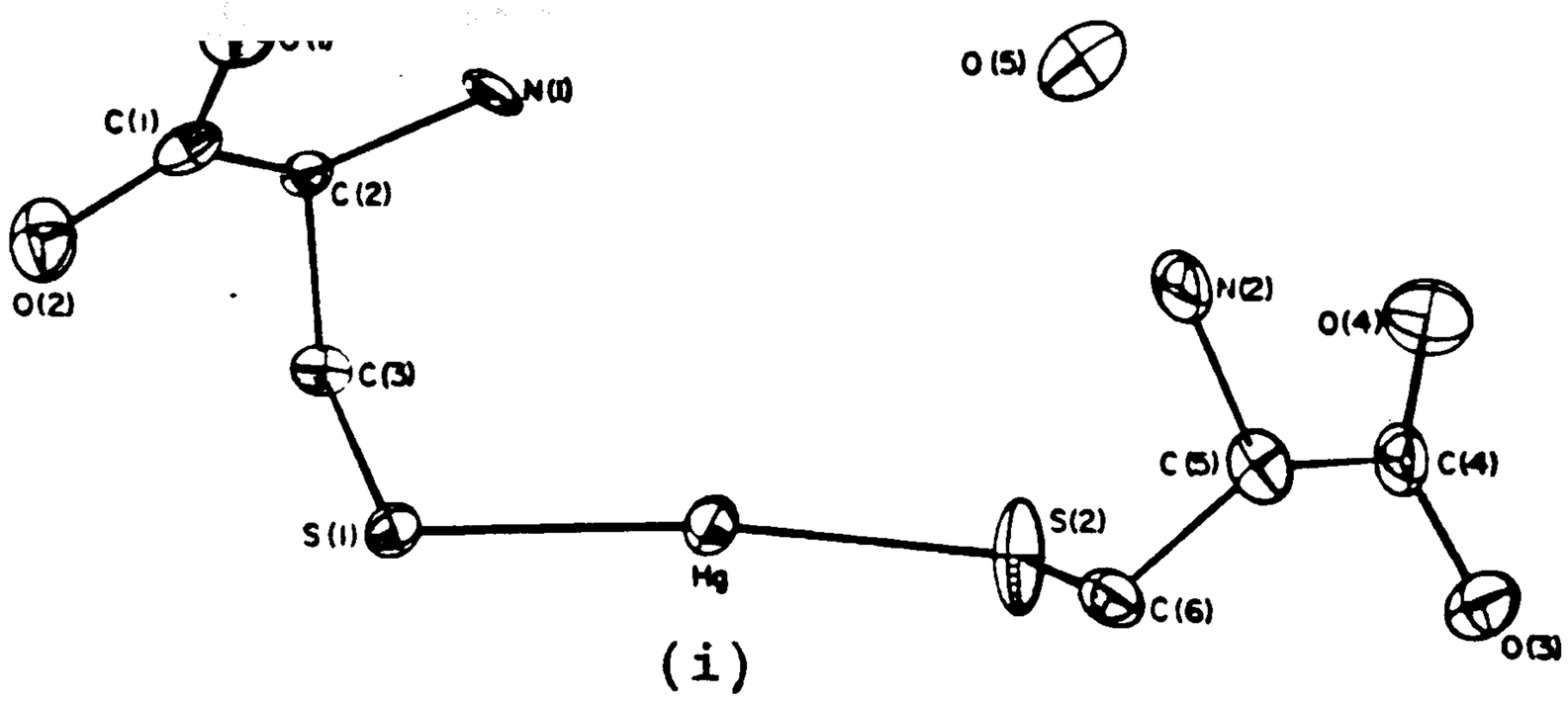
However, the adsorption of mercury on suspended solids is probably the primary reason for the prevalence of particulate forms of mercury in natural waters[174]. In particular, the organic content of suspended matter has been shown to have a very large sorption ability for mercury[175]. This can be explained by the higher solubility of certain mercury species in organic matter than in water, and by

the formation of surface complexes with organics containing sulphur groups[176]. Molecules containing sulphur groups are present in the aquatic and sediment environment in the form of proteinaceous material; this material is generated by micro- and macrobiota and vegetation. The capacity of the polarisable Hg (II) ion to form strong complexes with donor atoms, such as sulphur, is well known; and sulphur may therefore be expected to play an especially important role in the environmental chemistry of mercury. There is, in fact, considerable evidence for the existance of environmentally significant Hg^{2+} complexes of the type RSHgX (X = anionic or neutral non-thiol ligand)[177,178].

Particulate material in water systems tends to settle out and become part of the bed sediment. Thus, association of mercury with particulates plays an important role both in the removal of mercury from waters and in determining the chemical form of mercury incorporated into bed sediments.

It may be assumed that mercury in the sediment environment is complexed largely to sulphide and sulphur groups. In the estuarine environment, where chloride concentrations are high, coordination of mercury to both chlorine and sulphur may be expected.

This chapter reports the formation of methylmercury in sediments incubated in the laboratory with model mercury compounds (these were prepared from reactions of mercury salts with sulphur-containing amino acids) and investigates the role of the chemical environment of mercury in controlling mercury methylation. The complexes used in this study are listed in Fig. 49, synthetic details for these compounds have been reported elsewhere[179,180,181,182]. In addition to the compounds listed in Fig. 49, incubation experiments were performed with mercuric chloride ($HgCl_2$), mercuric acetate ($Hg(CH_3CO_2)_2$) and mercuric sulphide



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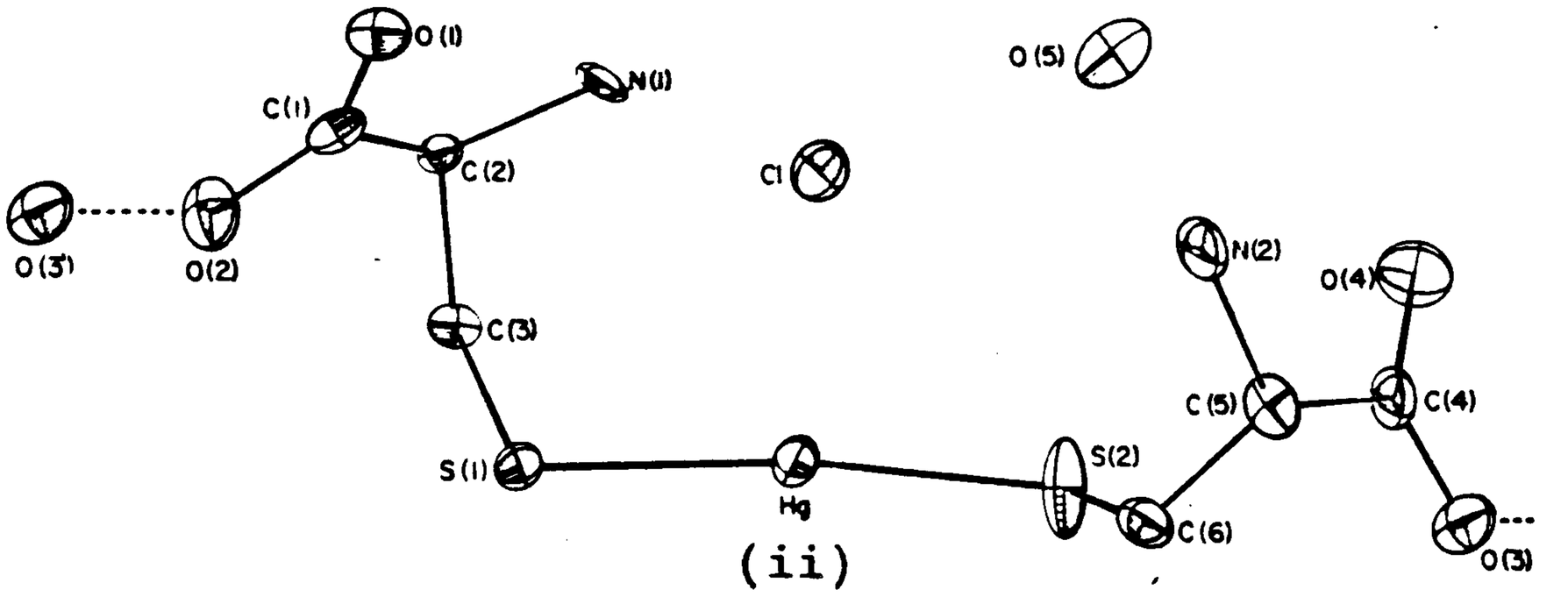

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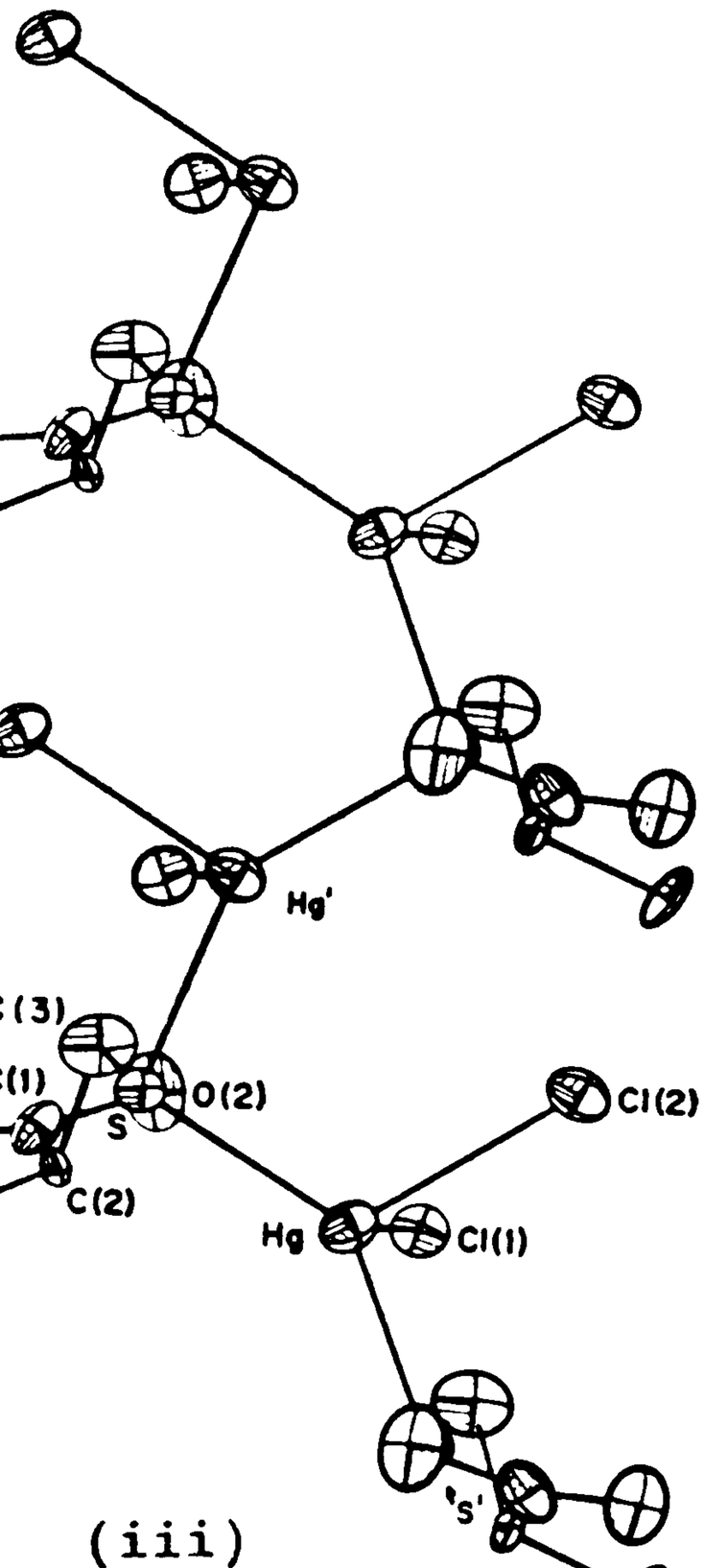

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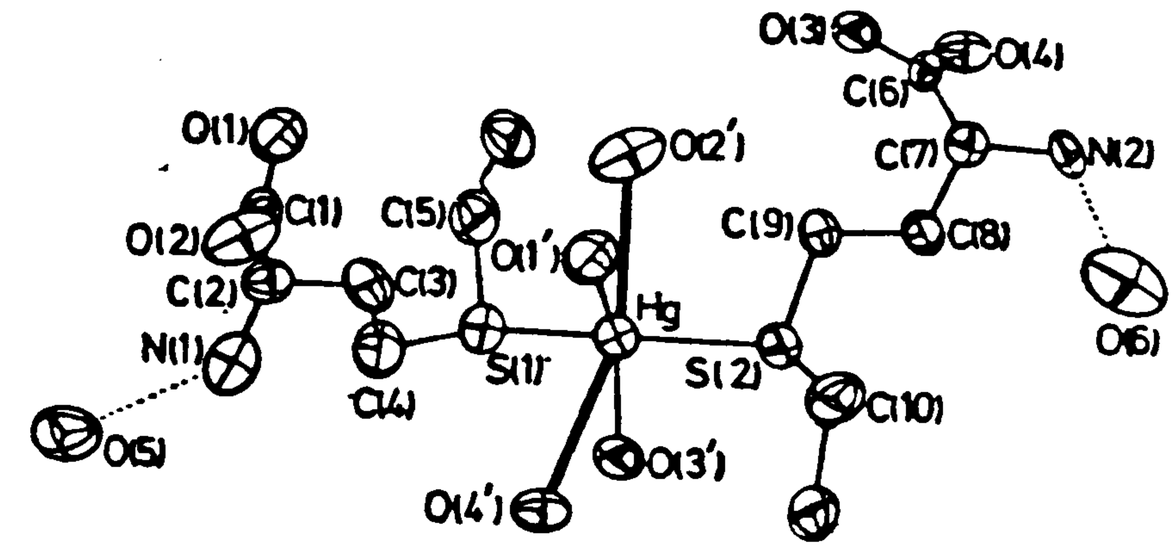

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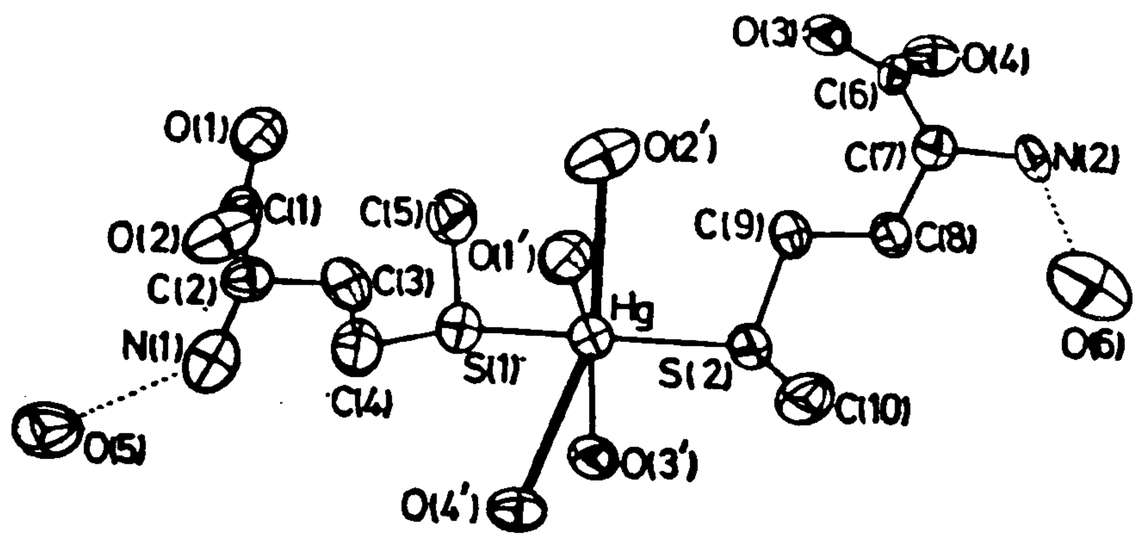

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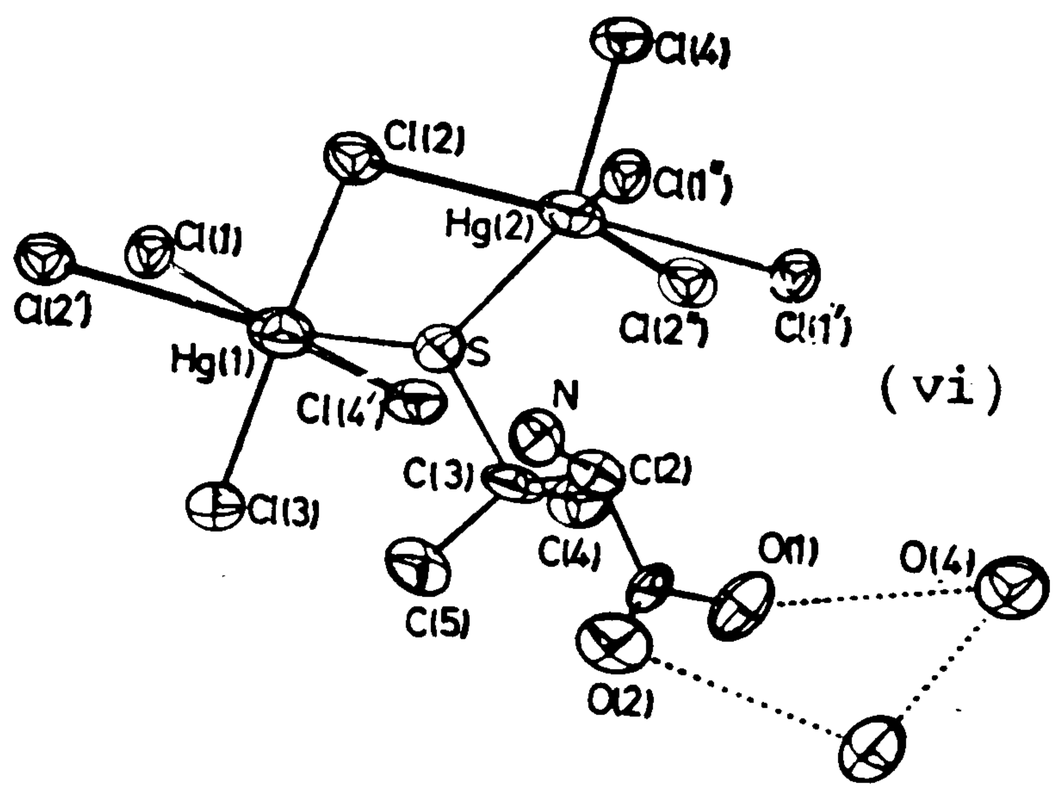


(vi)

Fig. 49.

(HgS). Solubility and bond length data for these compounds are presented in Table 17. The compounds were incubated in homogeneous sediments obtained from an inter-tidal location in the Dart estuary. The chemical parameters of the sediments were as follows: Eh = -70 mV, sulphide content = 0.57 mg g^{-1}, methylmercury content = 1.2 ng g^{-1}, TOC = 3.7 %, total mercury = 0.2 ug g^{-1}.

Method

Samples of the complexes (5 x 10^{-4} moles with respect to mercury) were weighed accurately into 250 cm^3 beakers. A 100 g portion of sediment was added to each of the beakers and the sediment/compound mixtures homogenised by stirring with a glass rod; the sediments were thus amended to the 100 ppm level with respect to mercury. The beakers were sealed with plastic film, to prevent the sediments from drying, and stored in the dark. Samples were withdrawn periodically from the beakers and analysed for methylmercury content by the method described in Chapter 8. An unamended sediment was incubated and analysed in parallel with the above to act as a control.

Results

Table 18 lists the methylmercury concentrations determined in the sediment/compound mixtures over a 40 day period. The values listed in Table 18 are corrected methylmercury levels obtained by subtracting the unamended sediment (which varied between 0.8 and 3.0 ng g^{-1}) from the levels of methylmercury determined in the sediment/compound mixtures. A plot of the data presented in Table 18 is shown in Fig. 50.

The highest rates of methylmercury production were observed in the $Hg(CH_3CO_2)_2$ amended sediment. Methylmercury may have formed in this sediment both as a result of reaction of $Hg(CH_3CO_2)_2$ with methylating agents present in

Table 17. Solubility and Bond Length Data

Compound	Bond Length (A)	Solubility ($g\ dm^{-3}$)
(1) $Hg[SCH_2CH(NH_3)COO]_2$ $Hg(Cyst)_2$	Hg-S 2.35	insoluble
(2) $Hg[SCH_2CH(NH_3)COOH][SCH_2CH(NH_3)CO_2]Cl.\frac{1}{2}H_2O$ $Hg(Cyst)_2Cl.\frac{1}{2}H_2O$	Hg-S 2.353 & 2.329 Hg-Cl 3.232	insoluble
(3) $HgCl_2[SCH_2CH(NH_3)COOH]$ $HgCl_2(Cyst)$	Hg-S 2.490 & 2.453 Hg-Cl 2.582 & 2.645	insoluble
(4) $Hg[EtSCH_2CH_2CH(NH_3)COO]_2[ClO_4]_2$ $Hg(eth)_2(ClO_4)_2$	Hg-S 2.500 & 2.475 Hg-O 2.27, 2.82 2.32 & 2.86	3
(5) $Hg[MeSCH_2CH_2CH(NH_3)COO]_2[ClO_4]_2$ $Hg(meth)_2(ClO_4)_2$	Hg-S 2.500 & 2.475 Hg-O 2.27, 2.82, 2.32 & 2.86	3
(6) $[HgCl_2]_2[SCMe_2CH(NH_3)CO_2]2H_2O$ $(HgCl_2)_2(pen).2H_2O$	Hg-S 2.356 & 2.822 Hg-Cl 2.687, 2.380, 2.780, 3.091, 3.426, 3.429, 2.356, 2.357, 2.850 & 3.323.	insoluble
$HgCl_2$	Hg-Cl 2.29	69
$Hg(CH_3COO)_2$		250
HgS	Hg-S 2.36	insoluble

cyst = cysteine, eth = ethionine, meth = methione, pen = penicillamine.

Table 18

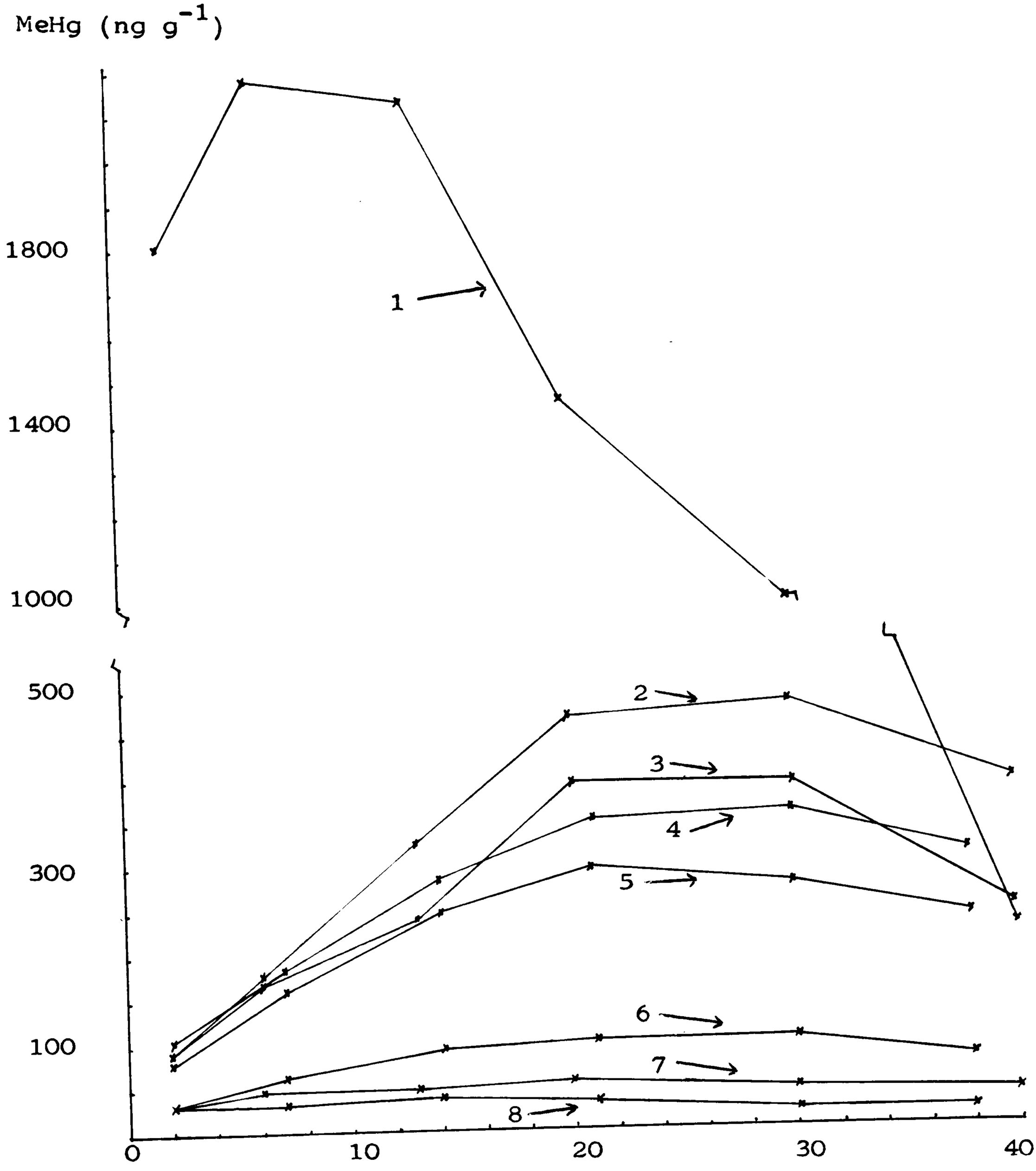
Methylmercury Levels in Incubated Sediments (ng g^{-1})

Compound	Incubation Period (days)					
	2	6	13	20	30	40
$HgCl_2$	28.3	42.5	43.7	55.3	45.2	41.6
$Hg(CH_3CO_2)$	1795.8	2191.2	2141.4	1462.3	1005.4	229.6
HgS	2.2	2.8	3.1	2.5	2.7	1.84
$Hg(cyst)_2Cl$	90.2	159.1	242.1	397.3	394.0	300.5
$Hg(cyst)_2$	89.2	175.3	326.4	470.2	485.9	392.8
	2	7	14	21	30	38
$HgCl_2$(cyst)	31.5	61.7	89.3	102.1	103.8	82.7
$(HgCl_2)_2$(pen)	32.7	32.4	38.4	31.1	20.4	22.6
$Hg(eth)_2(ClO_4)_2$	78.7	159.9	251.5	298.1	278.2	242.3
$Hg(meth)_2(ClO_4)_2$	100.8	183.1	287.6	352.1	361.8	315.2

the sediment, and perhaps also by intra-molecular reaction, involving methyl migration from carbon to mercury within the $Hg(CH_3CO_2)_2$ molecule. The formation of methylmercury from $Hg(CH_3CO_2)_2$ has been reported previously by Akagi[88].

The HgS amended sediment produced low but definite yields of methylmercury; although it is possible that the small amounts of methylmercury produced may have been formed from mercury salt impurities in the HgS reagent.

If bond length is taken as a criterion of bond strength, and reactivity by the SN_2 mechanism, then the results show that the rates of methylmercury production in the amended sediments are not determined by the strength of mercury bonding in the complexes. Production rates of methylmercury are also found to be unrelated to the solubility of the



Key:-

1. $Hg(CH_3CO_2)_2$
2. $Hg(cyst)_2$
3. $Hg(cyst)_2Cl$
4. $Hg(meth)_2(ClO_4)_2$
5. $Hg(eth)_2(ClO_4)_2$
6. $HgCl_2(cyst)$
7. $HgCl_2$
8. $(HgCl_2)_2(pen)$

Fig. 50.

Results of Differential Incubation Experiments

compounds.

The results show that chlorine bonding to mercury has an inhibiting effect on methylmercury production. This may be understood if the breakdown of the complexes by microorganisms - leading to the formation of Hg^{2+} or Hg(O) perhaps - is necessary before mercury methylation can occur. It is possible that the anaerobic microorganisms in the sediment possess a greater capacity to breakdown sulphur-bonded-mercury-amino-acid complexes relative to complexes of mercury with sulphur-bonded amino acids and chlorine. This may be understood in the following way. Anaerobic bacteria derive energy by reducing sulphur; and sulphur atoms in the molecules of the complexes may act as electron acceptors in this process. The reduction reaction proceeds enzymatically and, therefore, involves the binding of an enzyme to the substrate. It is possible that enzymes produced by the microorganisms will be compatible with the amino acid part of the mercury-complex molecules and bind to it; however, chlorine groups may hinder or prevent this binding due to steric or electrostatic effects. Therefore, the release of reactive Hg^{2+} and Hg(O) species may proceed at a slower rate in sediments amended with mercury-amino-acid-chlorine complexes relative to sediments containing simpler complexes of mercury with amino acids only.

Mercuric chloride is toxic to microorganisms. The toxicity of the compound arises from direct binding of mercury on to enzymes, resulting in inhibition of their function[183]. The formation of methylmercury in the mercuric chloride amended sediment may have resulted from chemical attack of mercuric chloride by natural methyl carbanion donors, e.g. $Me(B_{12})$. The ability of $Me(B_{12})$ to methylate mercuric chloride directly has been demonstrated previously[77]. Mercuric sulphide also is a powerful inhibitor of enzymes. However, mercuric sulphide is immune to attack by methylating agents, such as $Me(B_{12})$ (see Chapter 17).

Interestingly, the sediments in which the highest rates of methylmercury production were observed also displayed a growth and decay pattern in methylmercury levels over the period of study, and this was most marked in the case of the mercuric acetate amended sediment (Fig. 50). For those sediments in which the rate of methylmercury production was comparatively low, a maximum methylmercury concentration was reached after $\sim$ 8 days, and this remained stable through out the course of study. A similar pattern of growth and stabilisation in methylmercury levels, in sediments amended with mercuric chloride to the 100 ppm level, has been reported by Jensen and Jernelov[8] (see Fig. 3)

Incubation Experiments with Sterilised Sediments

Experiments were performed to assess the extent of methylmercury production in sterilised sediments following inoculation with various mercury compounds. The sediments used in this series of experiments were homogeneous sediments, similar to those used in the previous experiment. The experimental procedure is described below.

Portions of sediment (100g) were weighed into a series of 250 cm^3 beakers. The beakers were covered with aluminium foil and autoclaved at 120^{o}C for 1 hour, thus effecting sterilisation of the sediments. The sediments were then amended, in a sterile glove box, with various mercury compounds to the 100 ppm level with respect to mercury. The sediment/compound mixtures were homogenised by stirring with a sterilised glass rod, following which the beakers were sealed with sterile plastic film and stored in darkness. After 7 days, the sediment/compound mixtures, along with an unamended sterilised sediment, were analysed for methylmercury content. Table 19 lists the residual methylmercury concentrations of the amended sediments following subtraction of the background methylmercury concentration (2.6 ng g^{-1}) of the control sediment.

Table 19. Methylmercury Levels in Sterilised Sediments After 7 days Incubation

Compound	MeHg (ng g^{-1})
$Hg(cyst)_2$	0.7
$Hg(cyst)_2Cl$	1.8
$HgCl_2(cyst)$	3.8
$(HgCl_2)_2(pen)$	3.7
$Hg(meth)_2(ClO_4)_2$	6.2
$Hg(eth)_2(ClO_4)_2$	5.3
$Hg(CH_3CO_2)_2$	2010.7
$Hg(CF_3CO_2)_2$	154.8
$Hg(ClO_3)_2$	50.8
$HgCl_2$	8.2
HgS	2.9

The results presented in Table 19 demonstrate the formation of only small amounts of methylmercury in all but the $Hg(CH_3CO_2)_2$, $Hg(CF_3CO_2)_2$ and $Hg(ClO_3)_2$ amended sediments.

The methylmercury concentration produced in the $Hg(CH_3CO_2)_2$ amended sediment is interesting in particular. Similar levels of methylmercury were formed after an incubation period of 6-7 days in both the $Hg(CH_3CO_2)_2$ amended sterile and non-sterile sediments (2010.7 and 2191.2 ng g^{-1} respectively). These results suggest that methyl migration in the $Hg(CH_3CO_2)$ molecule, not mediated by biological processes, is the major route to methylmercury formation in both sediments.

The other results presented in Table 19 point to a direct relationship between the degree to which mercury compounds dissociate in solution and the extent to which these compounds produce methylmercury on incubation in sterile

sediments. Dissociation constants for the mercury compounds under the experimental conditions used are not available, but qualitatively will increase in the following order: Hg-cysteine and Hg-penicillamine complexes < $Hg(eth)(ClO_4)_2$ < $HgCl_2$ < $Hg(ClO_3)_2$ < $Hg(CH_3CO_2)_2$ < $Hg(CF_3CO_2)_2$ (184,185, 186). The mercuric ions formed on dissociation of the compounds may be attacked chemically by methylating agents in the sediment. Although some of these methylating agents may be produced by biological processes, there is evidence to suggest that they form at a rate which ensures that a continuous supply is present. Therefore, following sterilisation of the sediment, mercuric ions may react with the methylating factor until the supply is exhausted. Definite, but reduced yields of methylmercury may, therefore, be produced in sterile sediments following incubation with mercury compounds which dissociate in aqueous systems to produce mercuric ions.

The small amounts of methylmercury produced in the sediments amended with the mercury-amino-acid complexes suggest that the biological decomposition of these compounds is necessary before methylation can proceed. The small yields of methylmercury produced in these sediments may have resulted from the methylation of mercuric ions produced by impurities in the reagents.

Chapter 17

An Investigation of Routes for Mercury Methylation in the Sediment Environment

Theoretical considerations concerning the speciation of mercury in the aquatic environment, which are outlined in the introduction of the previous chapter, suggest that mercury may be present in estuarine sediments mainly in the form of elemental mercury, mercuric sulphide and complexes in which mercury is coordinated to both sulphur and chlorine groups; additionally, mercury may form anionic complexes with chlorine, e.g. $HgCl_3^-$ and $HgCl_4^{2-}$, in saline environments. This chapter reports the results of model experiments which investigate new routes by which these compounds may be methylated in the sediment environment. The work is described in three sections.

(1) The results of experiments reported Chapter 16 indicate HgS is immune to attack by methylating agents and that the formation of methylmercury in sediments amended with mercury complexes proceeds after the complexes have been decomposed by microbiota. This work naturally leads to the investigations reported below.

Direct methylations of HgS and mercury complexes were attempted with natural methylating agents. Three methylating agents which produce different methyl species were selected for these experiments. These were methylcobalamin ($Me(B_{12})$),which normally transfers methyl carbanions; betaine ($Me_3NCH_2CO_2H$), which transfers methyl carbonium ions; and iodomethane (MeI), which transfers methyl carbonium ions by oxidative addition, or methyl radicals on exposure to light[187]. It should be noted that $Me(B_{12})$ may also produce methyl radicals on exposure to light, and, additionally, reactions of $Me(B_{12})$ involving transfer of a methyl group to a nucleo-phile are known[188]; although it is doubtfull if $Me(B_{12})$ is capable of methylating Hg(O)

by methyl carbonium ion transfer.

(2) There are no reports of the methylation of Hg(0) by betaine or $Me(B_{12})$. Such methylations would be important as Hg(0) may be present in the aquatic environment[170] contiguous to the two naturally occuring methylating agents. Therefore, experiments were performed in which Hg(0) was incubated with betaine and $Me(B_{12})$ and the formation of methylmercury investigated. The methylation of Hg(0) by MeI in a pure reaction system was originally demonstrated by Maynard[189] 50 years ago. However, there are no reports of this reaction occuring in the sediment environment. Therefore, experiments were undertaken to investigate the feasibility of a methylation of Hg(0) by MeI in a sediment matrix.

(3) Rodgers[89] and Nagase et al.[91] demonstrated the methylation of mercury (in the form of mercuric nitrate) by unspecified methylating agents present in the fulvic acid fraction of soils and sediments. The work of these authors was extended, and the capacity of Hg(0), HgS and mercury complexes to undergo methylation on incubation with fulvic acid solution, obtained from an estuarine sediment, was investigated.

(1) <u>Reactions of Mercuric Sulphide and Mercury Complexes with Methylcobalamin, Betaine and Iodomethane</u>

(i) Small amounts (100 mg) of HgS and other mercury complexes (Table 17) were weighed into test-tubes. To each tube, 5 cm^3 of a $Me(B_{12})$ solution (conc. 5×10^{-5} mol dm^{-3}) were added, and the mixtures incubated in darkness (to avoid decomposition of the light sensitive $Me(B_{12})$). After 7 days, the mixtures were shaken, and a 4 cm^3 aliquot of supernatent liquid was withdrawn from each tube and analysed for methylmercury content. Reagent blanks were incubated and analysed in parallel. The results of these experiments are presented over the page.

Compound	Yield of Methylmercury (moles)
HgS	N.D.
$Hg(cyst)_2$	N.D.
$Hg(cyst)_2Cl\frac{1}{2}H_2O$	N.D.
$HgCl_2(cyst)$	N.D.
$(HgCl_2)_2(pen)$	N.D.
$Hg(meth)_2(ClO_4)_2$	2.37×10^{-7}
$Hg(eth)_2(ClO_4)_2$	2.257×10^{-7}

N.D. = not detected above the blank level.

The yields of methylmercury from $Hg(meth)_2(ClO_4)_2$ and $Hg(eth)_2(ClO_4)_2$ were close enough to the theoretical maximum (2.5×10^{-7} moles) to be considered 100 per cent within the limits of experimental error. Methylmercury was not detected above the blank level (<5 ng) in any of the other reaction mixtures.

(ii) Small amounts (100 mg) of HgS and other mercury complexes were weighed into test-tubes. To each tube, 0.5 cm^3 (1.14 g) of MeI was added. The mixtures were incubated for 7 days and then analysed in parallel.

Results: Methylmercury was not detected above the blank level (<5ng) in any of the reaction mixtures.

(iii) Similar amounts of HgS and the mercury complexes to those used in (i) and (ii) were weighed into test-tubes. To each tube, 5 cm^3 of a betaine solution (conc. 5×10^{-2} mol. dm^{-3}) were added, and the mixtures incubated for 7 days. At the end of this period, the mixtures were shaken and a 4 cm^3 aliquot of supernatent liquid was withdrawn from each tube and analysed for methylmercury.

Results: Methylmercury was not detected above the blank level (<5 ng) in any of the reaction mixtures.

The results of these experiments show that HgS and the

mercury-cysteine and mercury- penicillamine complexes resist attack by methyl carbanions, methyl radicals and methyl carbonium ions. However, the mercury-methione and mercury-ethionine complexes are methylated by methyl carbanion attack; reactions of these compounds with $Me(B_{12})$ are discussed further in Chapter 18.

(2) Reaction of Elemental Mercury with Methylcobalamin, Betaine and Iodomethane

(i) A small amount of Hg(0) (20 ul ≡ 0.27 g) was introduced into a test-tube along with 5 cm^3 of a $Me(B_{12})$ solution (conc. 5 x 10^{-5} mol. dm^{-3}), and the mixture incubated in darkness. After 7 days, the mixture was shaken, and 4 cm^3 of the supernatent liquid was withdrawn from the tube and analysed for methylmercury. Reagent blanks were incubated and analysed in parallel.

Result: Methylmercury was not detected above the blank level (<5 ng).

(ii) A small amount (0.27 g) of Hg(0) was introduced into a test-tube along with 5 cm^3 of a betaine solution (conc. 5 x 10^{-2} mol. dm^{-3}). The mixture was incubated for 7 days and then 4 cm^3 of the supernatent liquid was withdrawn from the tube and analysed for methylmercury. Reagent blanks were incubated and analysed in parallel.

Result: Methylmercury was not detected above the blank level (<5 ng).

(iii) Two 50 g portions of a homogenous sediment, obtained from the Dart estuary, were inoculated with 0.37 ul (5 mg) of Hg(0) and 22 ul (50 mg) of MeI. The sediments thus were amended to the 100 ppm level with respect to Hg(0) and 1000 ppm level with respect to MeI. Two separate 50 g portions of sediment were amended to the 100 ppm level with respect to Hg(0), and two further 50 g portions of sediment were amended with MeI to the 1000 ppm level. These

sediments, together with two 50 g portions of unamended sediments, were incubated for 7 days and then analysed for methylmercury content. The results are presented below.

Sediment	Methylmercury Content
Hg(O) + MeI	4.2 & 5.1 ng g^{-1} (dry wt.)
Hg(O)	4.6 & 5.5 " "
MeI	3.2 & 4.0 " "
Unamended	3.4 & 3.8 " "

Discussion

The failure of the last experiment to demonstrate the methylation of Hg(O) by MeI in a sediment matrix may have resulted from poor mixing of the reactants. Hg(O) was added to the sediment as a small globule (0.37 ul capacity) and this could not be dispersed throughout the sediment. The mercury remained in the sediment as a few small globules even after vigorous stirring, and thus only a fraction of Hg(O) added to the sediment could interact with it. The reaction of Hg(O) with photolysis products of MeI, which may have been produced at the surface of the sediment, also was improbable. Maynard[189] stated that unless Hg(O) was in a finely-divided state, the methylation of the element by MeI was dependent upon the reaction of Hg(O) with iodine, liberated photochemically from MeI, as a first step. The mercurous iodide formed in this reaction is decomposed photochemically, producing a finely-divided form of Hg(O) which is methylated by MeI. The reaction sequence is shown below:

$$2Hg + I_2 \longrightarrow Hg_2I_2$$

$$Hg_2I_2 \longrightarrow HgI_2 + Hg^*$$

$$Hg^* + MeI \longrightarrow MeHgI$$

The result of experiment (i) demonstrates the inability of $Me(B_{12})$ to methylate Hg(O). This result is not

surprising, as Hg(0) possesses a lone pair of electrons in its outer shell, and is therefore unlikely to behave as an electrophile towards carbanions. However, the formation of methylmercury from the reaction of Hg(0) with betaine, experiment (ii), may be expected, as the lone pair of electrons on Hg(0) may enable it to behave as a nucleophile towards carbonium ions. The absence of methylmercury in the reaction solution of experiment (ii) may be explained by the necessity for Hg(0) to be in a reactive form before methylation by carbonium ions can take place, as discussed above.

The electron deficient Hg^{2+} and covalent Hg(II) would not be expected to behave as nucleophiles towards carbonium ions, and, indeed, incubations of $Hg(CF_3CO_2)_2$ and $HgCl_2$ with betaine and MeI failed to produce methylmercury.

(3) Reactions of Mercury and Mercury Compounds with Fulvic Acid Obtained From An Estuarine Sediment

The following procedure[191] was used to prepare fulvic acid from a sediment obtained from an intertidal location in the Dart estuary. A 500 g portion of wet sediment was mixed with 1 dm^3 of an aqueous sodium hydroxide solution (conc. 0.5 mol dm^{-3}) and the mixture filtered. The filtrate was acidified to pH 1 with hydrochloric acid, and fractionated into humic acid precipitate and fulvic acid solution by centrifugation. Finally, the supernatent liquid was amended with sodium hydroxide to pH 7, whereupon the fulvic acid extract was ready for use.

Small amounts (100 mg) of Hg(0), HgS, $HgCl_2$, $Hg(CF_3CO_2)_2$ and the mercury complexes were weighed into beakers. Fulvic acid solution (100 cm^3) was added to each beaker, and the mixtures incubated. After 7 days, 20 cm^3 aliquots of supernatent liquid were withdrawn from each beaker and analysed for methylmercury content. Reagent blanks were incubated and analysed in parallel. The amounts of

methylmercury that were produced in the reaction mixtures are presented below:

Compound	Methylmercury
$Hg(cyst)_2$	N.D.
$Hg(cyst)_2Cl\frac{1}{2}H_2O$	N.D.
$HgCl_2(cyst)$	N.D.
$(HgCl_2)_2(pen)$	N.D.
$Hg(meth)_2(ClO_4)_2$	121 ng
$Hg(eth)_2(ClO_4)_2$	107 ng
HgS	N.D.
Hg(O)	15 ng
$HgCl_2$	105 ng
$Hg(CF_3CO_2)_2$	5381 ng

N.D. = not detected above the blank level (<5 ng).

This series of experiments was repeated using sterile fulvic acid solution. This was obtained by autoclaving a portion of the fulvic acid which had been prepared for the previous experiment, at 120°C for 1 hour. The results of the experiments are presented below:

Compound	Methylmercury
$Hg(cyst)_2$	N.D.
$Hg(cyst)_2Cl.\frac{1}{2}H_2O$	N.D.
$HgCl_2(cyst)$	N.D.
$(HgCl_2)_2(pen)$	N.D.
$Hg(meth)_2(ClO_4)_2$	117 ng
$Hg(eth)_2(ClO_4)_2$	128 ng
HgS	N.D.
Hg(O)	N.D.
$HgCl_2$	98 ng
$Hg(CF_3CO_2)_2$	5132 ng

N.D. = not detected above the blank level (<5 ng).

The results of both sets of experiments demonstrated low but definite yields of methylmercury from 4 of the mercury compounds. In addition, a small amount of methylmercury was produced from the incubation of Hg(0) with non-sterile fulvic acid solution, although incubation of Hg(0) in sterile fulvic acid solution failed to produce methylmercury. Sterilisation of the fulvic acid solution did not alter significantly the amounts of methylmercury produced by the 4 compounds. The largest amount of methylmercury was obtained from $Hg(CF_3CO_2)_2$; mean yield = 5256 ng, which is equivalent to a yield of 0.006 per cent with respect to mercury. The relatively high yield of methylmercury obtained from $Hg(CF_3CO_2)_2$ may have resulted from the greater extent to which the compound dissociates in aqueous solution relative to $HgCl_2$, $Hg(meth)_2(ClO_4)_2$ and $Hg(eth)_2(ClO_4)_2$. The results, therefore,indicate that the methylation of mercury by fulvic acid is an abiotic process, and proceeds by transfer of methyl carbanions. The small amount of methylmercury generated from incubation of Hg(0) in non-sterile fulvic acid solution may have resulted from biological conversion of a small amount of Hg(0) to Hg(II).

Conclusions

The results of the experiments described in sections 1, 2 and 3 are summarised in Table 20.

The following conclusions may be drawn from results of experiments described in this chapter:-

(1) The insoluble mercury-cysteine and mercury-penicillamine complexes resist attack by methylating agents. Therefore, the chemical or biotic decomposition of these compounds is necessary for methylmercury to be produced from these complexes inthe sediment environment. This reasoning also applies to HgS.

(2) Soluble Hg(II) compounds are attacked by methyl carbanions, and the methylation of Hg^{2+} proceeds at a faster

Table 20. Methylation of Mercury

Compound	Methylating Agents				
	$Me(B_{12})$	MeI	Betaine	Fulvic acid (non-sterile)	Fulvic acid (sterile)
$Hg(cyst)_2$	-	-	-	-	-
$Hg(cyst)_2Cl.\frac{1}{2}H_2O$	-	-	-	-	-
$HgCl_2(cyst)$	-	-	-	-	-
$(HgCl_2)_2(pen)$	-	-	-	-	-
$Hg(meth)_2(ClO_4)_2$	+	-	-	+	+
$Hg(eth)_2(ClO_4$	+	-	-	+	+
HgS	-	-	-	-	-
Hg(O)	-	I	-	+	-
Hg(O) in sed. matrix	N.A.	-	N.A.	N.A.	N.A.
$HgCl_2$	I	-	-	+	+
$Hg(CF_3CO_2)_2$	+	-	-	+	+

+ = methylmercury detected, - = methylmercury not detected,
N.A. = not attempted, I = not attempted but known from previous work to produce methylmercury.

rate than the methylation of covalent Hg(II).

(3) Hg(O) is not readily attacked by methyl carbonium ions. The methylation of Hg(O) by carbonium ions may proceed only if the element is in a finely-divided or reactive form.

Chapter 18

Kinetic Studies of Reactions Between Methylcobalamin and Mercury-Methionine and Mercury-Ethionine Complexes

The formation of methylmercury from reactions of $Hg(meth)_2(ClO_4)_2$ and $Hg(eth)_2(ClO_4)_2$ ($Hg(meth)_2^{2+}$ and $Hg(eth)_2^{2+}$ in aqueous solution at neutral pH) with $Me(B_{12})$ was reported in Chapter 17. This chapter reports the results of kinetic studies of these reactions. The studies were performed by measuring the increase in concentration of aquocobalamin, $H_2O(B_{12})^+$ - the product formed when $Me(B_{12})$ transfers a methyl group in aqueous solution - in the reaction mixtures with time. The U.V. spectrum of $H_2O(B_{12})^+$ (Fig. 51) contains a strong absorption band centred at 350 nm; this absorption band is absent from the U.V. spectrum of $Me(B_{12})$ (Fig. 52). The formation of $H_2O(B_{12})$ was monitored, therefore, by measuring the increase in absorbance of the reaction mixtures at 350 nm.

Preliminary Experiments

These experiments were designed to determine suitable concentrations for the reactants, identify the reaction products and determine their yields.

Reagents:-

(i) $Me(B_{12})$ 5×10^{-5} mol. dm^{-3} aqueous. This reagent was prepared in a dark room and stored in an opaque vessel to prevent conversion of the light sensitive $Me(B_{12})$ to $H_2O(B_{12})$.

(ii) mercury-methionine 10^{-2} mol. dm^{-3} aqueous. This was prepared as follows: Two m moles of methionine (0.2980g) were dissolved in $\sim$50 cm^3 of distilled water. One m mole of mercuric oxide (0.2166 g) was added to the solution along with 1 cm^3 of perchloric acid (60 %). The mixture was stirred to dissolve the mercuric oxide, and the

Fig. 51.

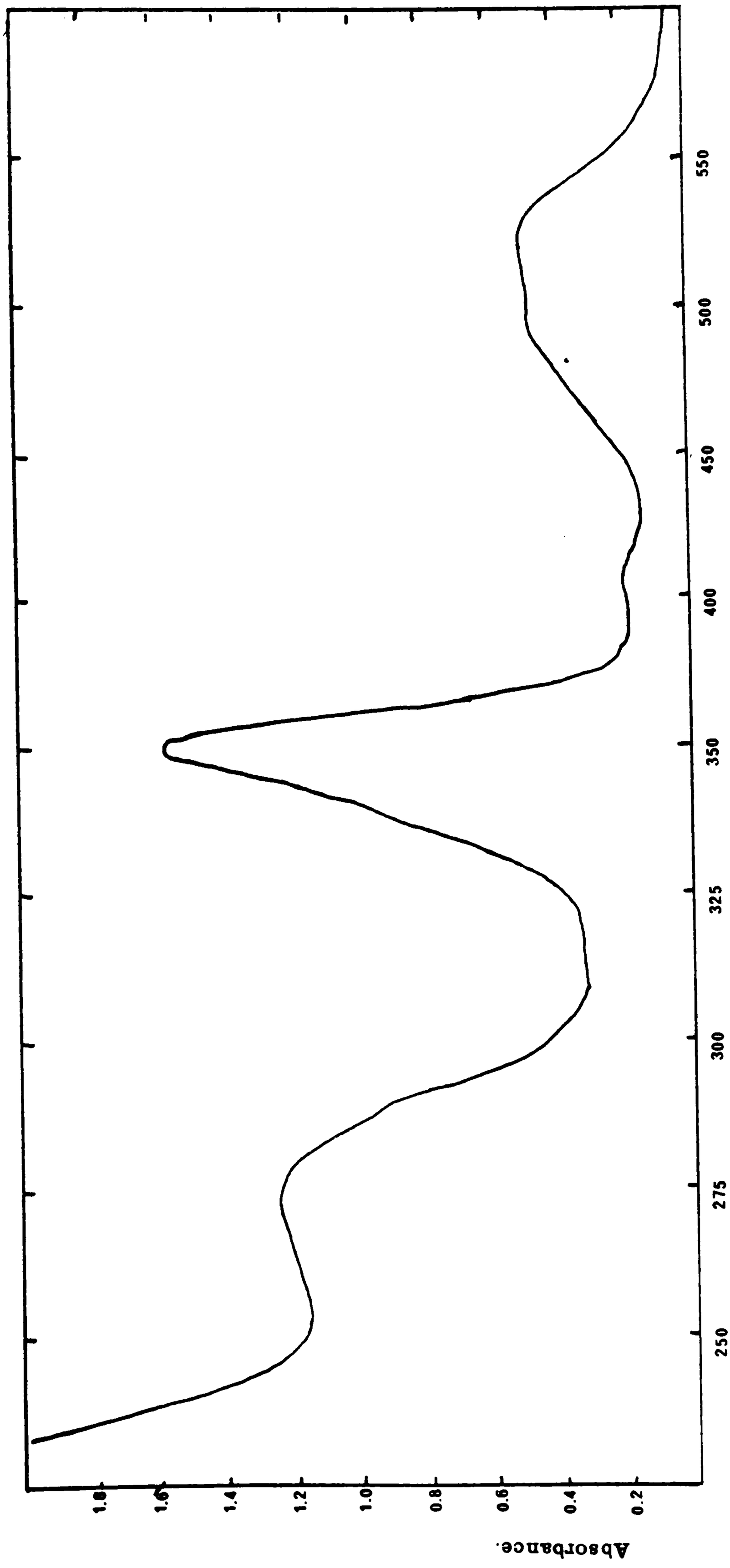
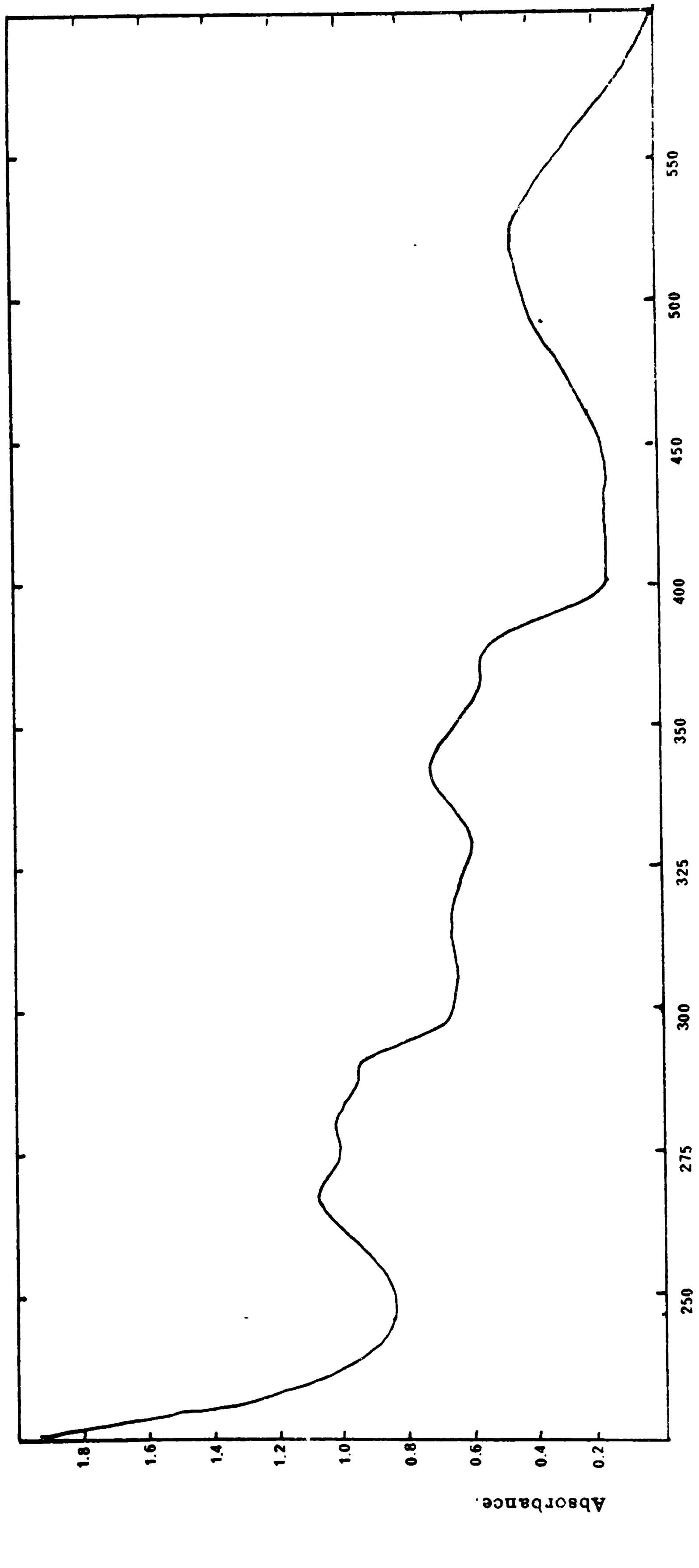

U.V. Spectrum of H_2O $(B_{12})^+$
Absorbance.
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
250
275
300
325
350
400
450
500
550
Wavelength (nm).

Fig. 52.



U.V. Spectrum of Me(B_{12})

solution volume then made up to 100 cm^3 with distilled water.

(iii) mercury-ethionine 10^{-2} mol dm^{-3} aqueous. This was prepared by the same procedure as that used to synthesise reagent (ii), except ethionine was substituted for methionine.

Instrumentation:

A Perkin-Elmer 555 u.v./vis. spectrophotometer was used in time mode to record increase in absorbance at 350 nm with time. A water bath accessory was attached to the spectrophotometer to maintain a constant temperature in the cell block.

Method

A 1 cm path-length Spectrosil cell was filled with distilled water and placed in the reference beam of the spectrophotometer. Meanwhile the water bath was set at 25°C and the flasks containing the reactants were immersed in the water. After temperature equilibration had been achieved, exactly 1.5 cm^3 of reagent (ii) was pipetted into a 1 cm path-length Spectrosil cell along with 1.5 cm^3 of $Me(B_{12})$ solution. The cell was stoppered, shaken, placed in the spectrophotometer and the increase in absorbance of the reaction mixture at 350 nm was monitored with time. The experimental procedure was repeated using reagent (iii).

Results and Discussion

The absorbance of the reaction solutions reached a steady value of~0.18 after 3 minutes. These reaction times were long enough to suggest that a full kinetic study of the reactions using conventional u.v./vis. techniques was feasible. The spectrophotometer was capable of measuring accurate absorbance values in the range 0-0.18, and thus an initial $Me(B_{12})$ concentration of 5×10^{-5} mol dm^{-3}

appeared to be suitable for kinetic experiments.

The reaction solutions were analysed for methylmercury contents by the method detailed in Chapter 8, and the $H_2O(B_{12})$ contents of the reaction solutions were calculated from the Beer-Lambert law, which states:

$$A = abC$$

where A is the absorbance of the solution, a is the absorptivity of $H_2O(B_{12})$, b is the path length of the cell and C is the concentration of $H_2O(B_{12})$ in the solution. The results are presented below:

	Amt. of $Me(B_{12})$ reacted (moles)	Amt. of $H_2O(B_{12})$ found (moles)	Yield of $H_2O(B_{12})$	Yield[(a)] of $MeHg^+$
$Hg(meth)^{2+}$	7.5×10^{-8}	7.34×10^{-8}	98%	94%
$Hg(eth)_2^{2+}$	7.5×10^{-8}	7.27×10^{-8}	97%	95%

[(a)] the yield of $MeHg^+$ was calculated as a percentage of the $Me(B_{12})$ taken.

Allowing for experimental error, the results of these experiments show that one molecule of $Me(B_{12})$ reacts with an excess of the mercury complexes to produce one molecule of $H_2O(B_{12})$ and one molecule of $MeHg^+$.

Kinetic Runs (First Series)

The study of the reactions under pseudo first order conditions with the mercury complexes in excess over $Me(B_{12})$ formed the second part of the investigation.

The reactions may be represented as shown below:

$$Hg[\text{amino acid}]^{2+} + Me(B_{12}) \xrightarrow{H_2O} MeHg^+ + H_2O(B_{12})^+$$

Now when the concentration of Hg-amino-acid is much greater

than that of $Me(B_{12})$, the change in concentration of Hg-amino-acid during the reaction is negligible and the reaction is said to be occurring under pseudo first order conditions. The rate of disappearance of $Me(B_{12})$ is then directly proportional to the concentration of $Me(B_{12})$ in the reaction mixture, and an observed rate constant can be defined as follows:

$$- \frac{d[Me(B_{12})]}{dt} = kobs[Me(B_{12})]$$

This rate expression can be integrated to give :

$$- \ln[Me(B_{12})] = kobs.t + c,$$

where c is the integration constant. At time t = 0, the concentration of $Me(B_{12})$ will be the initial concentration, $[Me(B_{12})]_o$. The integrated rate equation can, therefore, be written as:

$$- \ln[Me(B_{12})] = kobs.t - \ln[Me(B_{12})]_o$$

This equation can be rearranged to the following form:

$$\ln\left(\frac{[Me(B_{12})]_o}{Me(B_{12})}\right) = kobs.t \qquad (1)$$

The reaction between $Me(B_{12})$ and Hg-amino-acid is followed by measuring the absorbance of the product, $H_2O(B_{12})^+$, at 350 nm. Now since the yield of $H_2O(B_{12})^+$ from $Me(B_{12})$ is 100 %, it follows that

$$[Me(B_{12})]_o = [H_2O(B_{12})^+]_\infty ,$$

where $[H_2O(B_{12})^+]_\infty$ is the concentration of $H_2O(B_{12})$ at the end of the reaction. It also follows that

$$[Me(B_{12})]t = [H_2O(B_{12})^+]_\infty - [H_2O(B_{12})^+]t$$

Now according to the Beer-Lambert relationship, the absorbance of $H_2O(B_{12})$ is directly proportional to its

concentration, i.e.,

$$\left[H_2O(\overset{+}{B}_{12})\right]_\infty \propto (A_{350})_\infty$$

$$\left[H_2O(\overset{+}{B}_{12})\right]t \propto (A_{350})t$$

It therefore follows that

$$\left[Me(B_{12})\right]_o \propto (A_{350})_\infty \qquad (2)$$

and $$\left[Me(B_{12})\right]t \propto (A_{350})_\infty - (A_{350})t \qquad (3)$$

substituting equations (2) and (3) into equation (1), the following equation is obtained:

$$\frac{\ln(A_{350})_\infty}{\ln\left[(A_{350})_\infty - (A_{350})\right]t} = kobs.t$$

Thus a plot of $\dfrac{\ln(A_{350})_\infty}{\ln\left[(A_{350})_\infty - (A_{350})\right]t}$ vs. t

should produce a straight line of slope, kobs.

Experimental

Reagents :-

$Me(B_{12})$ 5×10^{-5} mol. dm^{-3} aqueous

Mercury-methionine, mercury-ethionine. The following concentrations of these complexes were prepared in 0.6% perchloric acid solution:

5×10^{-2} mol. dm^{-3}
1×10^{-2} " "
5×10^{-3} " "
2.5×10^{-3} " "
1×10^{-3} " "
5×10^{-4} " "

Method

The procedure described on p. 140 was followed through for each of the mercury-amino-acid solutions listed above. A typical spectrum produced by these experiments is shown in Fig. 53.

Results

From the absorbance vs. time spectra, values for $(A_{350})_\infty$ and $(A_{350})t$, and hence $\ln\left[\frac{(A_{350})_\infty}{(A_{350})_\infty - (A_{350})t}\right]$, were calculated. This is illustrated below for the results obtained from the reaction solution containing an initial mercury-methionine concentration of 2.5×10^{-2} mol. dm^{-3}; the data is presented in Table 21, and the corresponding graph in Fig. 54.

Table 21. Results of a Kinetic Run

t(seconds)	$(A_{350})t$	$\ln \frac{(A_{350})_\infty}{(A_{350})_\infty - (A_{350})t}$
6	0.088	0.755
12	0.113	1.142
18	0.130	1.528
24	0.142	1.934
30	0.149	2.279
∞	0.166	-

The slope of the graph, calculated by the method of least squares, gives a value of 6.4×10^{-2} s^{-1} for kobs.

The values of kobs. obtained from the reaction solutions containing various concentrations of mercury-methionine and mercury-ethionine are presented in Tables 22 and 23.

Fig. 53.

Trace Obtained from a Kinetic Run

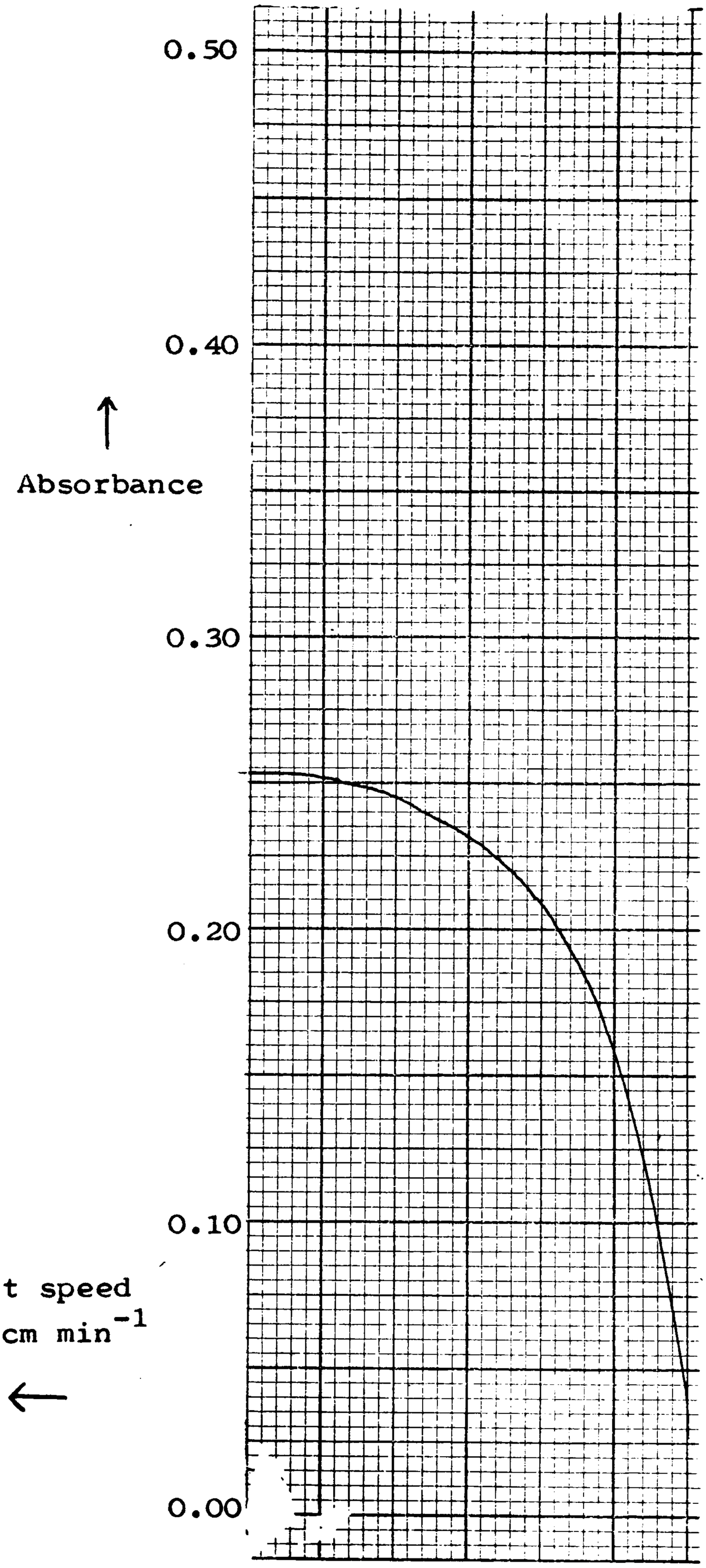


Fig. 54.

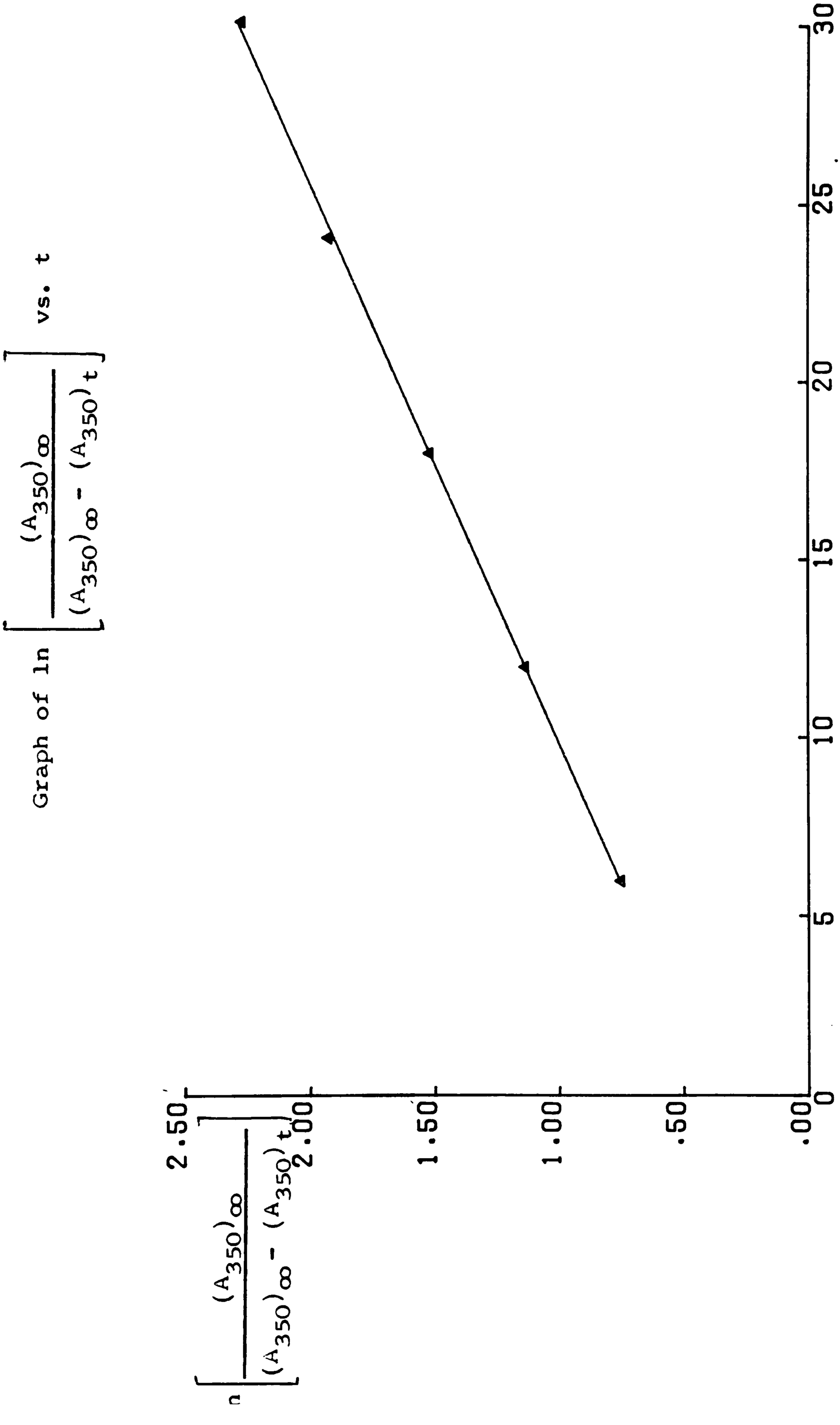

Graph of ln [(A350)∞ / ((A350)∞ - (A350)t)] vs. t
ln [(A350)∞ / ((A350)∞ - (A350)t)]
2.50
2.00
1.50
1.00
.50
.00
0
5
10
15
20
25
30
t(secs.)

Table 22. kobs. Values for Reaction Solutions Containing Mercury-methionine

[Mercury-methionine] (mol. dm^{-3})	kobs. (s^{-1})
2.5×10^{-2}	6.40×10^{-2}
5×10^{-3}	4.12×10^{-2}
2.5×10^{-3}	3.67×10^{-2}
1.25×10^{-3}	2.72×10^{-2}
5×10^{-4}	2.25×10^{-2}
2.5×10^{-4}	1.75×10^{-2}

Table 23. kobs. Values for Reaction Solutions Containing Mercury-ethionine

[Mercury-ethionine] (mol. dm^{-3})	kobs. (s^{-1})
2.5×10^{-2}	7.39×10^{-2}
5×10^{-3}	4.07×10^{-2}
2.5×10^{-3}	3.38×10^{-2}
1.25×10^{-3}	2.81×10^{-2}
5×10^{-4}	2.06×10^{-2}
2.5×10^{-4}	1.65×10^{-2}

Discussion

With the concentrations of the mercury-amino-acid complexes in excess over that of $Me(B_{12})$, the rates of the reactions are described by Equation (4).

$$\text{Rate} = \text{kobs}[Me(B_{12})] \qquad (4)$$

However, the true rate constant for the reactions, k, is defined by Equation (5).

$$\text{Rate} = k[\text{Me}(B_{12})][\text{Hg(amino acid)}]^n \qquad (5)$$

where n is the order of the reaction with respect to the mercury complex. A comparison of Equations (4) and (5) relates kobs and k as follows:

$$\text{kobs} = k[\text{Hg(amino acid)}]^n \qquad (6)$$

Equation (6) can be written in a logarithmic form, as shown below:

$$\ln\text{kobs} = \ln k + n\ln[\text{Hg(amino acid)}]$$

Thus a plot of lnkobs vs $\ln[\text{Hg(amino acid)}]$ should produce a straight line of slope n, the order of the reaction with respect to the mercury compound.

Plots of lnkobs vs $\ln[\text{Hg(amino acid)}]$ for the data presented in Tables 22 and 23 are shown in Figs. 55 and 56 respectively. Least squares analyses of the data give values of n = 3.57 and 3.33, for the order of the reactions with respect to mercury-methione and mercury-ethionine respectively.

For mechanistically simple reactions, experimentally determined orders are usually small inte gers. However, the fractional orders reported here suggest that the reactions between $Me(B_{12})$ and the mercury compounds are mechanistically complex.

Kinetic Runs (second series)

The first series of kinetic runs were carried out with reaction solutions containing 0.6 per cent perchloric acid (the pH values of the solutions were 1.2). The reactions were carried out at low pH in order to obtain kobs values over a wide range of Hg-amino-acid concentrations: the low solubility of the complexes at pH 7 restricted the concentration range which could be studied under neutral conditions. However, as fractional n values had been obtained

Fig. 55.

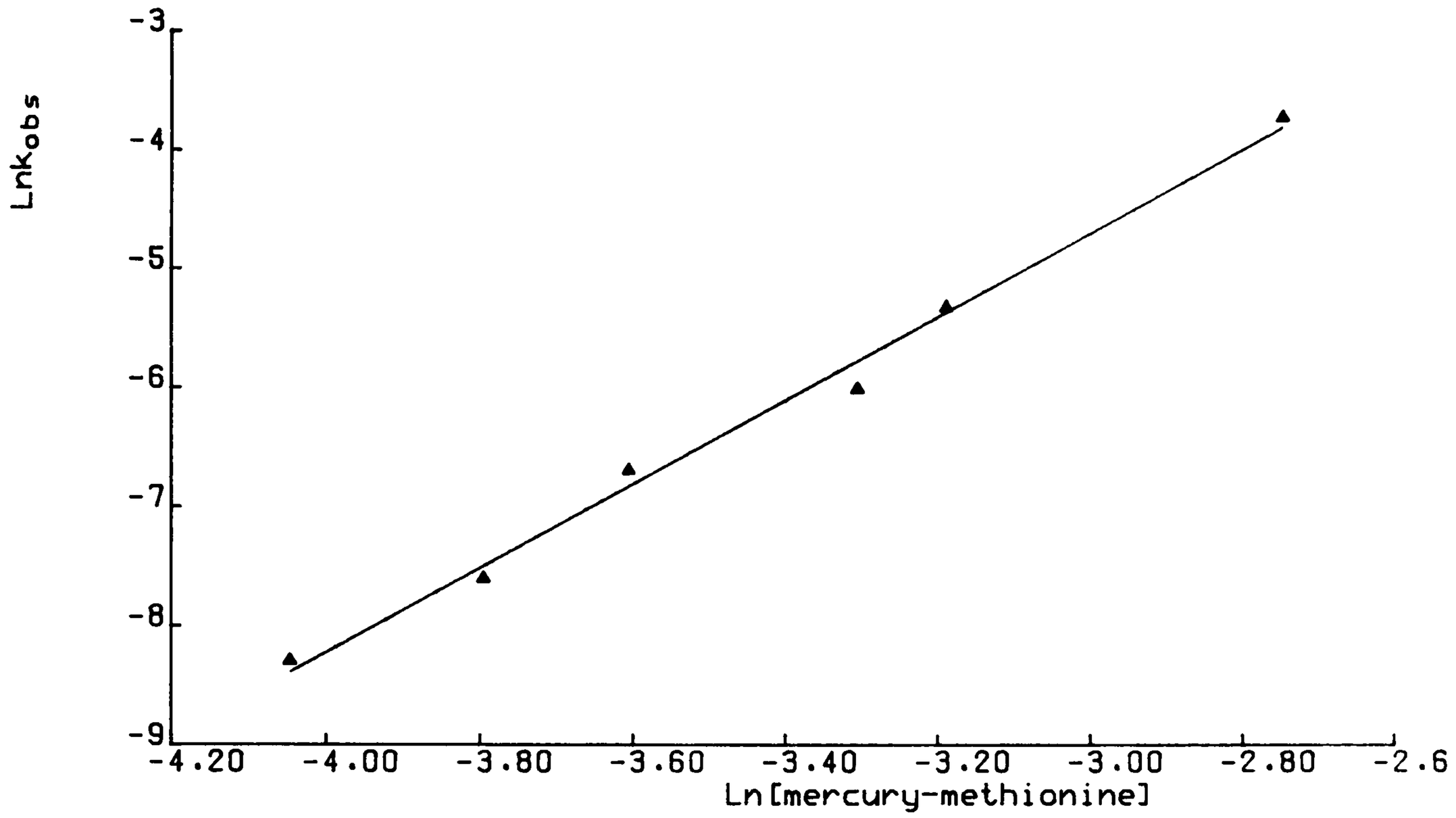
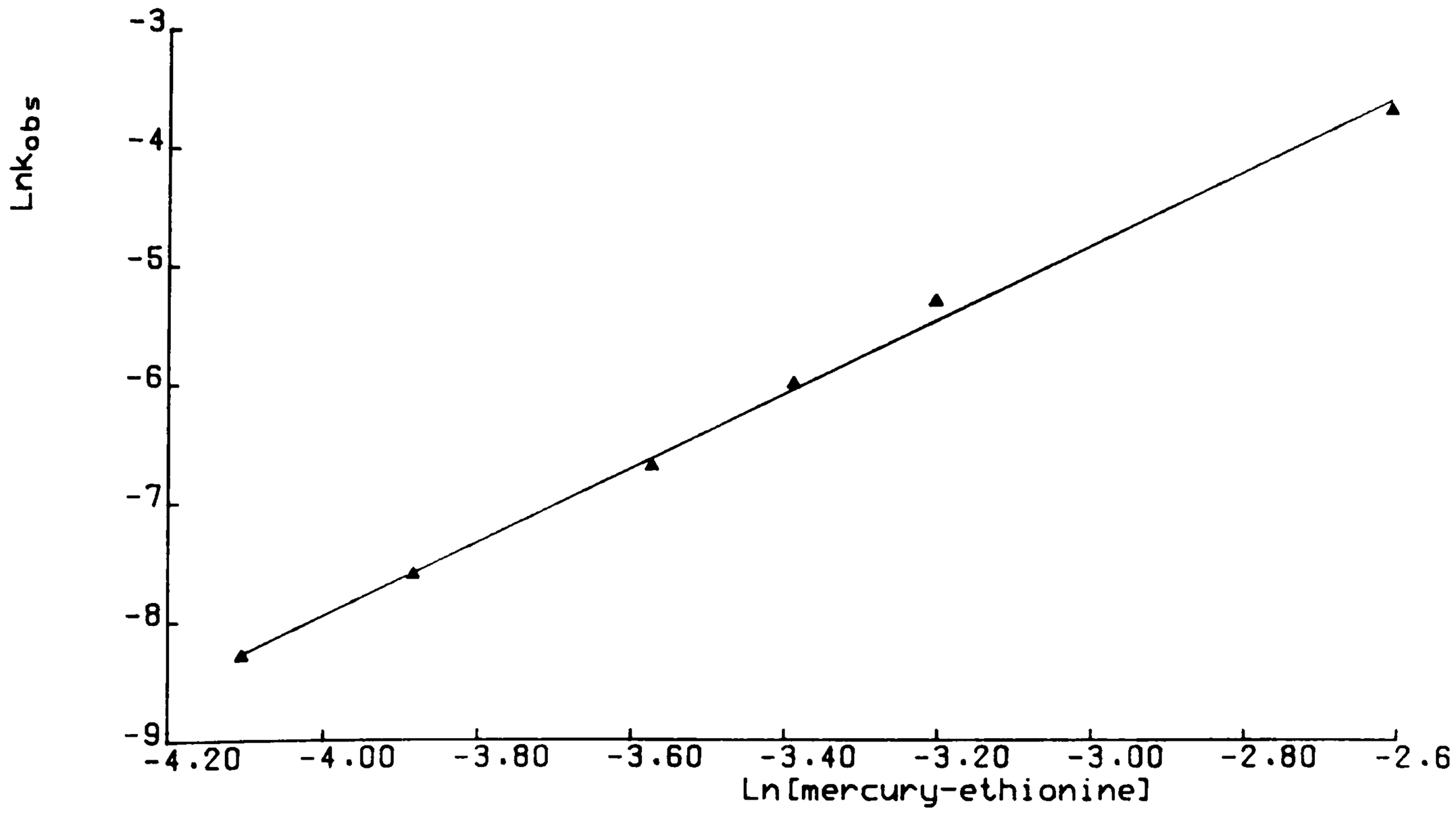

Lnk$_{obs}$ vs Ln[mercury-methionine]
Lnk$_{obs}$
-3
-4
-5
-6
-7
-8
-9
-4.20
-4.00
-3.80
-3.60
-3.40
-3.20
-3.00
-2.80
-2.6
Ln[mercury-methionine]

Fig. 56.


Lnk$_{obs}$ vs Ln[mercury-ethionine]
Lnk$_{obs}$
-3
-4
-5
-6
-7
-8
-9
-4.20
-4.00
-3.80
-3.60
-3.40
-3.20
-3.00
-2.80
-2.6
Ln[mercury-ethionine]

in the first series of kinetic runs, these studies were repeated over a narrower concentration range with solutions buffered to higher pH values, in order to ascertain if the mechanism of the reactions simplified on moving from acidic to alkaline conditions.

Experimental

Reagents:-

$Me(B_{12})$ 5×10^{-5} mol. dm^{-3} prepared in pH 7 buffer. Mercury-methione, mercury-ethionine. Solutions containing 5×10^{-2} mol. dm^{-3} of these compounds were prepared by the procedure outlined on p 140. Aliquots of 10, 7.5, 5, 2 and 1 cm^3 of these solutions were pipetted into 100 cm^3 volumetric flasks and the volumes made up to the mark with pH 7 buffer. Thus solutions of the following concentrations were prepared:

5×10^{-3} mol. dm^{-3}
3.75×10^{-3} mol dm^{-3}
2.5×10^{-3} mol dm^{-3}
1×10^{-3} mol dm^{-3}
5×10^{-4} mol dm^{-3}

Method

The procedure described on page 140 was followed through for each of the mercury-amino-acid solutions listed above. The pH values of the reaction solutions were also checked and all were found to be at pH 7.

Results

The kobs. values obtained from the kinetic runs are presented in Tables 24 and 25.

Plots of ln[Mercury-methionine] and ln[mercury-ethionine] vs kobs. are straight lines; the values for the slopes of these lines, calculated by the method of least squares, are presented below:

Table 24. kobs. Values for Reaction Solutions Containing Mercury-methionine

[Mercury-methionine]	kobs.	ln [Mercury-methionine]	ln kobs.
2.5×10^{-3} mol dm^{-3}	3.658×10^{-3} s^{-1}	- 5.9915	- 5.6108
1.875×10^{-3} " "	2.799×10^{-3} s^{-1}	- 6.2791	- 5.8786
1.25×10^{-3} " "	2.008×10^{-3} "	- 6.6846	- 6.2109
5×10^{-4} " "	7.004×10^{-4} "	- 7.6009	- 7.2639
2.5×10^{-4} " "	4.746×10^{-4} "	- 8.2940	- 7.6530

Table 25. kobs. Values for Reaction Solutions Containing Mercury-ethionine

[Mercury-ethionine]	kobs.	ln [Mercury-ethionine]	ln kobs.
2.5×10^{-3} mol dm^{-3}	4.863×10^{-3} s^{-1}	- 5.9915	- 5.3260
1.875×10^{-3} " "	3.613×10^{-3} "	- 6.2791	- 5.6231
1.25×10^{-3} " "	2.667×10^{-3} "	- 6.6846	- 5.9266
5×10^{-4} " "	9.062×10^{-4} "	- 7.6009	- 7.0062
2.5×10^{-4} " "	6.183×10^{-4} "	- 8.2940	- 7.3885

ln mercury-methionine vs. kobs : slope (n) = 0.92

ln mercury-ethionine vs. kobs : slope (n) = 0.93

The n values of 0.92 and 0.93 are near enough to the theoretical value of 1.0 for simple second order processes to be considered as reasonable within the limits of experimental error. Alternatively, these values could imply mechanistic complexity for the reactions leading to a real divergence from simple second order rate equations. The reaction between Me(B_{12}) and one of the complexes, mercury-methione, was therefore studied at three other temperatures to obtain further data. Values of kobs were measured at 48°C, 36°C and 9°C; the results are presented in Table 26.

The following values for the orders of the reaction with respect to mercury-methione at various temperatures are obtained from a least squares analysis of the data presented in Table 26:

Temperature	Order of reaction (n)
48°C	0.84
36°C	0.84
24°C	0.92
9°C	0.93

These results indicate a divergence away from simple first order kinetics with respect to mercury-methione as the reaction temperature rises.

Attempted Studies at pH4 and pH9

Attempts were made to study the reactions between Me(B_{12}) and the mercury-amino-acid complexes in solutions buffered to pH 4 and 9. However, at pH 4 the rates of reaction were too fast for kinetic studies to be made using conventional u.v./vis. techniques. Conversely, at pH 9, Me(B_{12}) failed to methylate the mercury compounds.

Table 26. Values of kobs. (s^{-1})

Temp. (^{o}C)	[Mercury-methionine] (mol. dm^{-3})			
	2.5×10^{-3}	1.25×10^{-3}	5×10^{-4}	2.5×10^{-4}
48	1.7013×10^{-2}	9.1600×10^{-3}	4.5433×10^{-3}	2.3825×10^{-4}
36	7.7521×10^{-2}	4.3100×10^{-3}	1.9758×10^{-3}	1.1421×10^{-3}
24	3.6583×10^{-3}	2.0075×10^{-3}	7.0040×10^{-4}	4.7460×10^{-4}
9	1.4377×10^{-3}	6.4650×10^{-4}	3.2170×10^{-4}	1.8050×10^{-4}

Discussion

The results reported in this chapter show that reactions of mercury-methione and mercury-ethione with $Me(B_{12})$ are kinetically complex, and both pH and temperature influence significantly rates and orders of the reaction. These effects are discussed below.

Effect of pH

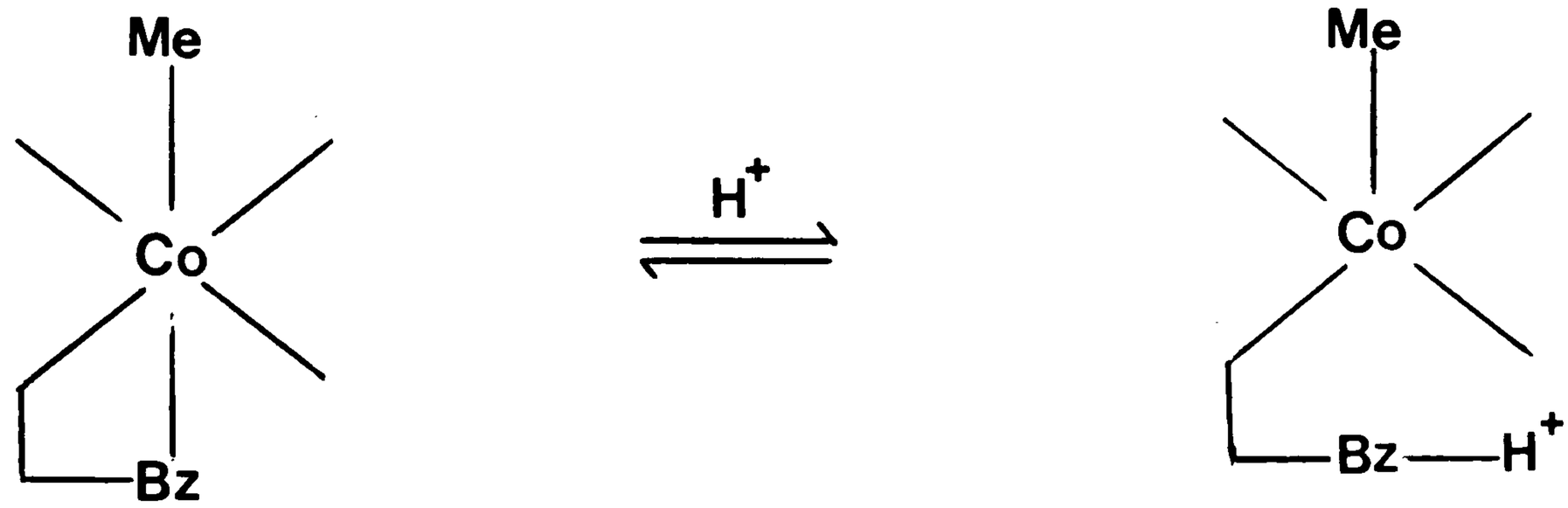
Slower rates of reaction between $Me(B_{12})$ and the mercury compounds are observed at pH 1.2 relative to pH 4.0. This observation results from the protonation of the benzimidazole ring in the $Me(B_{12})$ molecule at low pH (Fig. 57). Displacement of the benzimidazole ring to form "base-off" $Me(B_{12})$ may serve to strengthen the Co-C σ bond, since electron density is withdrawn from Co and the electrons in the Co-C bond are held more tightly, making electrophilic displacement of CH_3^- more difficult. At pH 1.2, $Me(B_{12})$ is present in solution entirely as the "base-off" form, whereas at pH 4 , $Me(B_{12})$ is present in solution primarilly as the reactive "base-on" species[192].

The failure of the mercury complexes to react with $Me(B_{12})$ at pH 9 results from deprotonation of $-\overset{+}{N}H_3$ groups in the complexes at this pH, and subsequent coordination of mercury to nitrogen. Thus, at pH 9 mercury is coordinated to oxygen, sulphur and nitrogen groups, and the complexes carry no residual charge (Fig. 58). Therefore, methylation of the complexes by $Me(B_{12})$ at high pH may be precluded by both steric hindrance of mercury and the reduced electrophilic nature of mercury, both of which factors may prevent displacement of CH_3^- from $Me(B_{12})$.

Faster rates for the reactions between $Me(B_{12})$ and the mercury complexes are observed at pH 1.2 relative to pH 7 (kobs. values are, on average, $\sim$20 x greater at the lower pH value). The faster rates of reaction result from protonation of $-COO^-$ groups in the complexes at low pH.

Fig. 57.

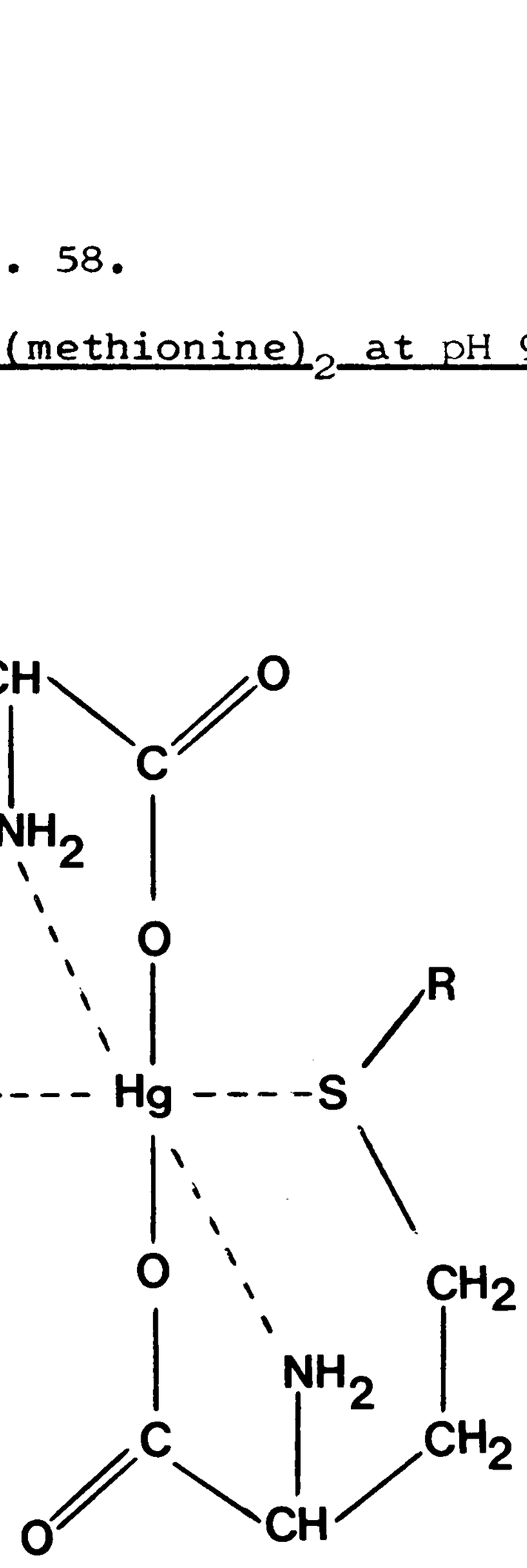
"Base-on" and Base-off Forms of $Me(B_{12})$



"base-on" "base-off"

Fig. 58.

The Structure of $Hg(methionine)_2$ at pH 9



Thus, at pH 1.2, the complexes may contain mercury coordinated primarily to sulphur groups only, and carry a charge of +4 (however, the fractional values obtained for the orders of the reactions at this pH suggest that more than one mercury species is methylated by $Me(B_{12})$). It is likely, therefore, that the reactions proceed at faster rates at pH 1.2 owing to reduced steric hinderence of mercury and the enhanced electrophilic nature of mercury; both of these factors make displacement of CH_3^- from $Me(B_{12})$ more amenable.

Effect of Temperature

The reactions between $Me(B_{12})$ and the mercury compounds at pH 7 diverge from second order kinetics with increasing temperature. This may be explained by increased dissociation of the complexes in solution at higher temperatures. The extent to which the complexes dissociate at various temperatures can be calculated from the Gibbs free energy equation, which states:

$$\Delta G = - RT\ln K,$$

Where ΔG is the change in free energy of reaction, R is the universal gas constant, T is the temperature of reaction and K is the equilibrium constant of the reaction. If it is assumed that ΔG values remain approximately constant over small temperature changes[193], then

$$-RT_1\ln K_1 = - RT_2\ln K_2$$

or

$$\frac{T_1}{T_2} = \frac{\ln K_2}{\ln K_1} \qquad (7)$$

where K_1 and K_2 are equilibrium constants for the reactions at temperatures T_1 and T_2 (K) respectively.

The following stability constant have been reported for the reaction between Hg^{2+} and methionine in KNO_3 solution

(conc. = 0.1 mol dm^{-3}) at $25^{o}C$[194].

$$Hg^{2+} + \text{methionine} \underset{}{\overset{k_1}{\rightleftharpoons}} Hg(\text{methionine})^{2+} \quad \log k_1 = 6.52$$

$$Hg(\text{methionine})^{2+} + \text{methionine} \underset{}{\overset{k_2}{\rightleftharpoons}} Hg(\text{methionine})_2^{2+} \quad \log k_2 = 4.93$$

On substititing the above values of k_1 and k_2 into Equation 7, the following values for the equilibrium constants of the reactions at $48^{o}C$ are obtained:

$$\log k_1 = 6.05, \qquad \log k_2 = 4.58$$

Therefore, the reaction between mercury-methionine and $Me(B_{12})$ may be expected to diverge from simple second order kinetics with increase in reaction temperature, owing to increased dissociation of the mercury complex.

Chapter 19

Reaction of Methylmercury with Sulphide in the Sediment Environment

Introduction

The loss of methylmercury from aqueous solutions of methylmercuric chloride following addition of hydrogen sulphide was demonstrated by Rowland et al.[195]. These workers reported that a solution of methylmercuric chloride (^{14}C labelled) treated with hydrogen sulphide showed an 80 % loss of radio-activity over 3 days. The group concluded that radio-activity was lost from the solution owing to the formation of a volatile sulphur derivative of methylmercury, although they were unable to identify this compound. Bartlett[103] extended the work of Rowland et al. and demonstrated the formation of bis (methylmercuric) sulphide, $(MeHg)_2S$, on mixing aqueous solutions of methylmercuric acetate and hydrogen sulphide. Bartlett further demonstrated the disproportionation of $(MeHg)_2S$ to mercuric sulphide and dimethylmercury. The complete reaction sequence is shown below.

$$2MeHg^{+} + S^{2-} \longrightarrow (MeHg)_2S \longrightarrow HgS + \uparrow Me_2Hg$$

Me_2Hg is an hydrophobic and volatile compound (boiling point = 96°C) and Rowland's [195] observation of the loss of radio-activity from solutions containing hydrogen sulphide and radio-labelled methylmercury is explained by the formation and volatilisation of the compound.

The hydrophobic and volatile nature of Me_2Hg suggests that this compound, once formed, would be lost rapidly from sediments. Therefore, reaction of methylmercury with sulphide, followed by dismutation of $(MeHg)_2S$, may be an important route leading to loss of methylmercury from the sediment environment.

Bartlett[103] was able to show that Me_2Hg was evolved into the gaseous phase from a sediment which had been inoculated with a large amount of methylmercury - to the 100 ug g^{-1} level - and then saturated with hydrogen sulphide. However, Bartlett was unable to detect the evolution of Me_2Hg from natural sediments following treatment with hydrogen sulphide, probably owing to the detection limitation (undefined by Bartlett) of the mass spectrometer/injection system used for the analysis of Me_2Hg in these experiments. Although Bartlett was unable to demonstrate the evolution of Me_2Hg from natural sediments, he was able to show that treatment of these sediments with hydrogen sulphide led to loss of methylmercury. In these experiments, hydrogen sulphide was passed through a container of freshly-sampled sediment; the sediment was divided into a series of bottles and analysed for methylmercury content over 28 days, and the results compared with those obtained from a control set of sediments which had not been exposed to hydrogen sulphide. Bartlett found that, on average, hydrogen sulphide reduced the level of methylmercury in the sediment by approximately 50 %.

A major aim of the present project was to define the role of the sulphide route of the Hg cycle, involving dismutation of $(MeHg)_2S$, in leading to loss of methylmercury from the sediment environment. Experiments, therefore, were performed in which natural sediments were incubated and the head space above the sediments analysed for methylmercury.

Experimental Work

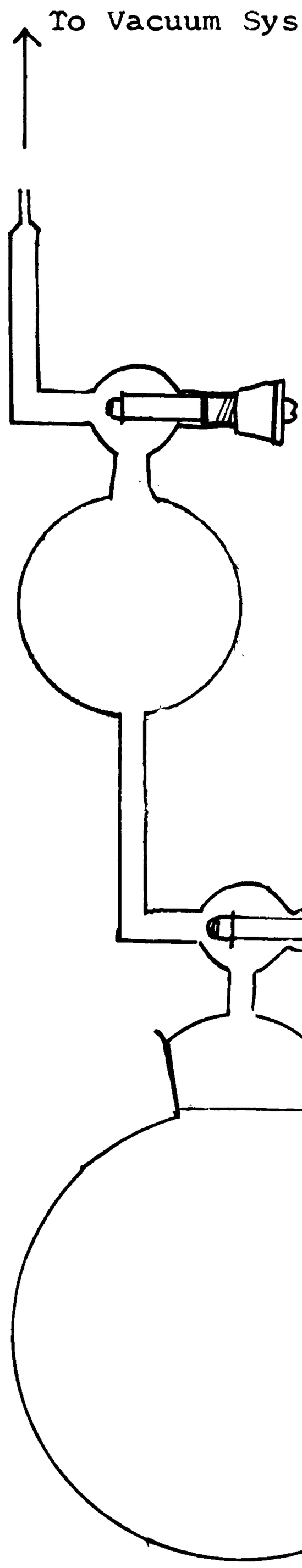
Apparatus:

A two chamber vessel (Fig. 59) was designed and constructed to collect volatile products formed during the incubation of sediment samples. The lower chamber (capacity = 5 dm^3) was filled with sediment, and volatile products were collected in the upper chamber (capacity = 2 dm^3).

Fig. 59.

Two Chamber Vacuum Vessel for

Collecting Me_2Hg Evolved from Sediments



Vacuum taps were incorporated between the lower and upper chambers of the vessel, and at the outlet of the upper chamber.

Instrumentation:

A Pye Unicam 204 gas chromatograph interfaced with a V.G. Micromass 16 F mass spectrometer was used to separate and detect volatile compounds evolved from sediment samples. The operational parameters for the chromatograph are listed below:-

Column type	: SCOT
Column length	: 50 m
Stationary phase	: OV 1
Column temp.	: 80^{o}C
Carrier gas	: helium

Method

A bulk sediment sample (~10 Kg) was collected at low water from an intertidal location in the Carron estuary (O.S map No. 65, ref. 905 823). The sample was homogenised in the laboratory, and subsamples withdrawn for analysis of water, sulphide and methylmercury content. The mean values obtained from the analysis of 5 replicate subsamples for each of the paramaters are shown below:

Water content	= 55.9 %
Sulphide content	= 1.7 mg g^{-1} (dry wt.)
Methylmercury content	= 58.2 ng g^{-1} (dry wt.)

Two portions of Carron sediment, one amended with sulphide to the 4 mg g^{-1} (dry wt.) level - a sulphide conc. at which the maximum point in the methylmercury/sulphide relationship is exceeded according to results reported in Chapter 13 - and one unamended with sulphide, were incubated, and the volatile products evolved from the sediments analysed for Me_2Hg. The procedure is described below.

The vessel shown in Fig. 59 was disconnected and 3.5 Kg of sediment, along with 500 cm^3 of distilled water, were added to the lower chamber. The vessel was reconnected and the air in the upper chamber pumped out into a vacuum system. When a vacuum had been established in the upper chamber, the tap at the outlet of the vessel was closed and tap between the lower and upper chambers opened. The sediment was incubated in daylight; occasionly the contents of the vessel were swirled to encourage any methylmercury which may have formed at the bottom of the sediment to rise to the surface. After an incubation period of 13 days (an incubation period shown previously to produce definite yields of Me_2Hg from methylmercury-amended sediments[103]) the tap connecting the lower and upper chambers of the vessel was closed. The contents of the upper chamber were then pumped out into a vacuum system along which a cold -temperature trap had been incorporated. The volatile compounds in the upper chamber were condensed into the trap using an ice/acetone mixture(temp $\sim$ -50°C).

The trap was connected to a 4-way valve at the inlet of the chromatograph, such that carrier gas could either by-pass the trap or flow into it. The trap was heated in a water bath to 100°C, and then the carrier gas was diverted into the trap and the contents of the trap swept on to the chromatographic column.

Results

The mass spectrometer did not detect Me_2Hg in the volatile products evolved from the unamended sediment. However, a small amount of Me_2Hg was detected in the volatile products which were evolved from the sediment which had been amended with sulphide. The quantity of Me_2Hg detected by the instrument was equivalent to an amount approximately 5 x the limit of detection (this was determined previously, from the injection of standards, as $\sim$ 20 ng). Therefore, approximately 100 ng of Me_2Hg had condensed in the cold trap.

The maximum theoretical yield of Me_2Hg from these experiments can be calculated as follows:

The amount of methylmercury present in the sediment (ng) =

wt. of sample (g) x solids content of sample (%) x methylmercury concentration (ng g^{-1} dry wt.)

$$= 3500 \times \frac{44.1 \times 58.2}{100} = 8.9832 \times 10^4 \text{ ng}$$

The equation on p.154 shows that 1 mole of Me_2Hg is produced from 2 moles of methylmercury. Therefore, the **maximum theoretical yield of Me_2Hg (ng) =**

$$\frac{89832}{\text{molec. wt. methylmercury}} \times \frac{\text{molec. wt. } Me_2Hg}{2}$$

$$= \frac{89832}{215.6} \times \frac{230.6}{2} = 4.8041 \times 10^4 \text{ ng}$$

The capacity of the head space above the sediment was ~ 3 dm^3, and 2 dm^3 of this was drawn through the cold trap. Therefore, if it is assumed that all Me_2Hg produced by the sediment became distributed evenly throughout the head space, the maximum amount of Me_2Hg which may have been detected

$$= 48041 \times \frac{2}{3} = 32027 \text{ ng}$$

The trapping efficiency of the method was assessed by injecting Me_2Hg standards into the vessel, and high recoveries (>90%) were obtained. Thus the percentage yield of Me_2Hg obtained from the sulphide amended sediment was approximately

$$\frac{100}{32027} \times 100 = 0.3\%$$

Finally, the methylmercury levels in the sediments were determined at the end of the incubation period. The mean values obtained from the analysis of 5 replicate subsamples

from each sediment are presented below.

Unamended sediment = 56.5 ng g^{-1}

Sulphide-amended sediment = 45.3 ng g^{-1}

Discussion

The results reported in this chapter can be summarised as follows:-

(1) The methylmercury level in the unamended sediment remained approximately the same after an incubation period of 13 days.

(2) Me_2Hg was not detected in the volatile products evolved from the unamended sediment.

(3) The methylmercury level in the amended sediment decreased significantly during the incubation period.

(4) A small amount of Me_2Hg was evolved into the head space above the sulphide amended sediment. About 100 ng of Me_2Hg, which is equivalent to a yield of ~0.3 % of the theore tical maximum, was detected by the mass spectrometer.

These points are discussed below.

(1) An increase in methylmercury content of the unamended sediment during the incubation period may have been expected in view of Morton's results which demonstrated the time dependency of methylmercury levels in sediments following sampling[102]. Indeed, the methylmercury levels in sediments collected from one location in the Carron estuary during the course of this project displayed a growth and decay effect (Chapter 11). However, the result of the present experiment, showing similar levels of methylmercury in the unamended sediment before and after incubation, implies (i) methylmercury was neither formed nor decomposed during the incubation period, or (ii) the rates of

methylmercury formation and decomposition in the sediment during incubation were similar. The latter suggestion does not preclude Me_2Hg formation in the sediment during incubation.

(2) The inability of the experiment to demonstrate the presence of Me_2Hg in the volatile products evolved from the unamended sediment implies (i) no Me_2Hg was produced or (ii) a small amount of Me_2Hg was produced in the sediment, but the amount evolved to the head space was less than the detection limit of the analytical method. The result indicates that no significant amount of Me_2Hg is formed in sediments which have low sulphide contents, either from biotic or abiotic methylation of methylmercury, or dismutation through the sulphide route.

(3) The methylmercury concentration of the sulphide - amended sediment decreased significantly; 22 % of the methylmercury content of the sediment was lost during the incubation period. This may have resulted from enhanced microbiological demethylating processes following inoculation of the sediment with sulphide.

The effect of sulphide concentration in depressing sediment methylmercury levels was investigated further. A series of sediments was amended with methylmercury to the 100 ng g^{-1} level, and then inoculated with various amounts of sulphide. The sediments were incubated for 5 days and then analysed for methylmercury content. The results obtained from a series of such sediments are summarised in Fig. 60; each point of the graph shown in Fig. 60 is a mean value obtained from the analysis of 5 individual sediment samples. The graph shows that sediment methylmercury and sulphide levels are related inversely over a sulphide concentration of at least 1-7 mg g^{-1}. The results reported in these experiments may be compared with Bartlett's[103] observation of a decrease in methylmercury levels of~50 % following saturation of sediments with H_2S.

LOSS OF METHYLMERCURY FROM SEDIMENTS INOCULATED WITH VARIOUS AMOUNTS OF SULPHIDE

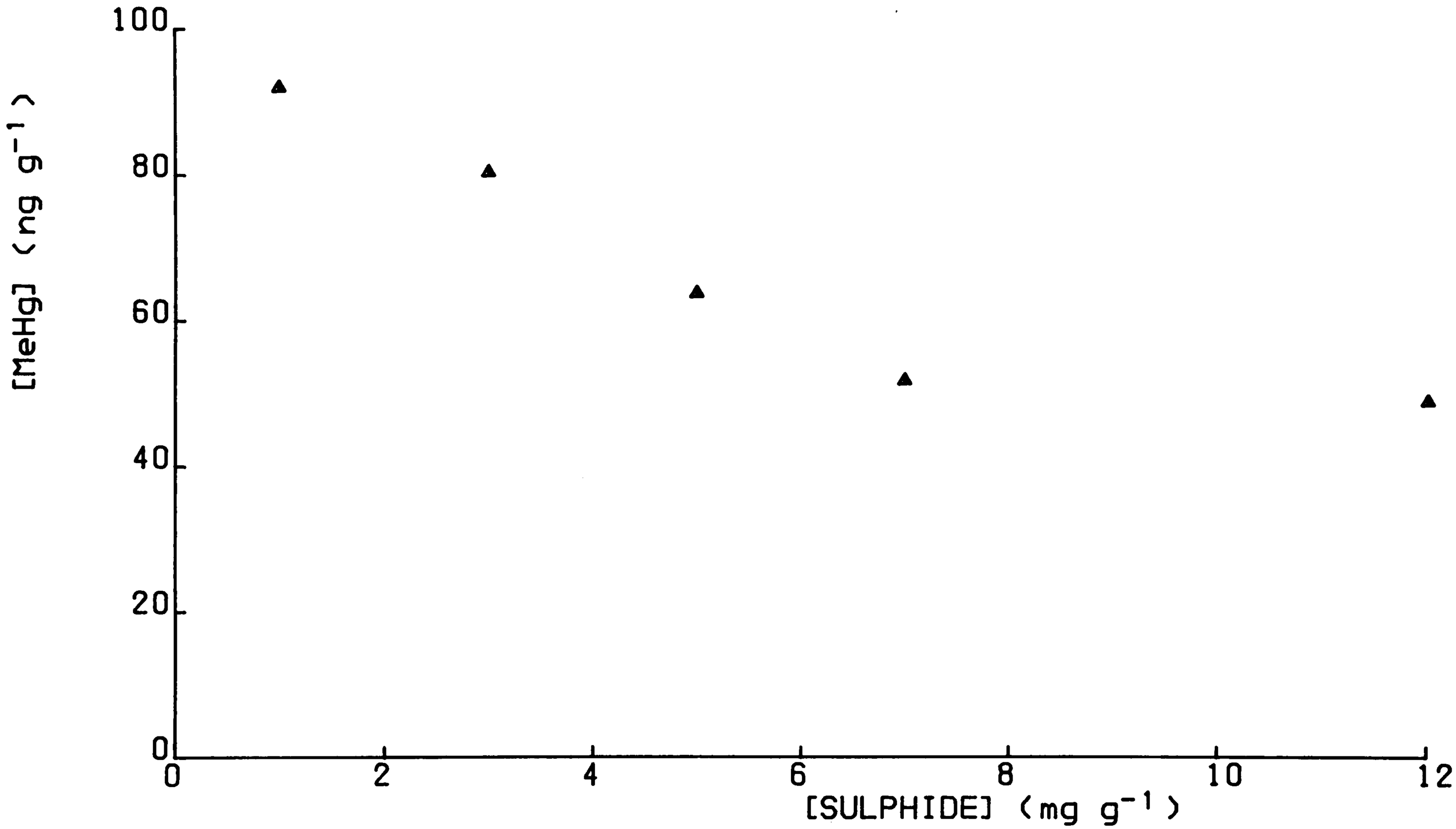


Fig. 60.

(4) The small amount of Me_2Hg (~100 ng) detected in the volatile products evolved from the sulphide-amended sediment does not correspond to the amount of Me_2Hg (10648 ng) which would have been produced had all the methylmercury lost from the sediment reacted via the scheme shown on P.154. There are 3 possible explanations for this observation : (i) only a small amount of Me_2Hg produced in the sediment volatilised into the head space; (ii) methylmercury reacted with sulphide, but only a small amount of $(MeHg)_2S$ formed dismutated - Bartlett[103] reported that $(MeHg)_2S$ is not detected by the analytical method employed for methylmercury determination; (iii) the addition of sulphide to the sediment promoted other loss mechanisms, biotic or abiotic, for methylmercury.

Finally, the question remains as to why methylmercury is present in the sediment environment, where sulphide concentrations (mg g^{-1} range) are invariably in large excess over those of methylmercury (ng g^{-1} range). This is perhaps best understood as being due to the formation of methylmercury complexes in the sediment environment which resist attack by sulphide. However, if methylmercury should be released from these complexes, as a result of a biological process for instance, then the higher the sulphide concentration in the sediment the greater the likelihood of reaction between methylmercury and sulphide occurring, with subsequent release of Me_2Hg. Another possible explanation is that the equilibrium constants for the reaction scheme shown on p.154 may be small at environmental temperatures. Evidence for the latter suggestion is to some extent provided by the work of Rowland et al.[195]. These authors reported that, at a reaction temperature of 37°C, 90 % of methylmercury was lost from aqueous solutions of methylmercuric chloride following addition of hydrogen sulphide, whereas at a reaction temperature of 5°C the loss of methylmercury from solutions treated with hydrogen sulphide was less than 50 %. Thus

a large excess of sulphide in the environment may be required to drive these reactions and produce significant quantities of Me_2Hg.

SECTION 5

FINAL DISCUSSION

Chapter 20

Overall Discussion

This chapter reviews the results reported in the various sections of the thesis. The inter-relaionships between these results and their implications for methylmercury production in the sediment environment are discussed.

The results may first be reviewed conveniently under the headings of Environmental Work and Synthetic Work; inter-relationships between the results reported under these headings are discussed later in the chapter.

Environmental Work

The results of each of the surveys undertaken during the course of the project demonstrated the importance of sulphide content as a factor controlling methylmercury levels in sediments. Methylmercury levels were found to rise directly with initial increase in sulphide concentration; although the results of the Carron and 1982 Clyde surveys demonstrated a maximum point in the methylmercury/sulphide relationship. From the results of all but one of the 10 surveys undertaken during the course of the work, methylmercury concentrations, in sediments with low sulphide contents, were found to correlate with sulphide concentrations at a higher level than any of the other sediment parameters that were measured; the results of the 1982 Clyde survey proved exceptional in that sediment methylmercury concentrations were found to be related more closely to total mercury levels. The existance of a strong linear relationship between methylmercury and sulphide levels in sediments with low sulphide contents may be attributed to 3 factors.

First, sulphide is present in the sediment environment largely as a result of the decay of organic material, and

therefore, sulphide levels in sediments may be expected to increase in line with organic matter concentration. Now as organic matter in the aquatic environment has a large affinity for mercury, the results are, in part, a reflection of the importance of total mercury concentrations in determining sediment methylmercury levels. The existence of a linear relationship between the latter two parameters is shown most clearly by the results of those surveys in which sediments covering a large range of total mercury levels are analysed, e.g. the Mersey and 1982 Clyde surveys.

Second, the rates of methylmercury formation and decomposition in the sediment environment are dependent upon the prevailing redox situation within the sediment. The range of sediment Eh values determined during the course of this project ranged from ~-350 to +250 mV, and these values fall within the "oxidising anearobic" zone defined by Billen[196] and Wollast[197]. Thus, the sediments are neither completely aerobic nor anaerobic and are able to accommodate a mixed microbiology. The importance of the latter point is that the peak metabol ic rates of the various aerobes and anearobes present in sediments occur under different redox conditions. Some bacteria with the capacity to methylate mercury have been shown to display peak metabol ic rates under anaerobic conditions, e.g. clostridia[143]. However, experiments with pure bacterial cultures have shown that aerobic conditions are generally more favourable to methylmercury formation. For instance, Vonk and Kaars Sijpesteijn[64] reported faster rates of mercury methylation by A. aerogenes and E. coli under aerobic relative to anaerobic conditions. Additionally, the authors demonstrated the methylation of mercury by the following bacteria under aerobic conditions: P. fluorescens, M. phlei, B. megaterium, E. Coli w/B_{12} and A. aerogenes w/B_{12}. Bisogni and Lawrence[73], working with bioreactor systems, have also reported faster methylation rates under

aerobic relative to anaerobic conditions. However, it has been shown that aerobic conditions also enhance rates of methylmercury decomposition. For instance, the demethylating species, Pseudomonas, was found to operate most efficiently in aerobic environments[63]. Additionally, Spangler et al.[69] found 30 bacterial cultures which could aerobically degrade methylmercury compared to 21 which could anaerobically degrade methylmercury. McCarty[71] also concluded that methylmercury was degraded faster by aerobic rather than anaerobic cultures. The results reported in this thesis indicated that net methylation rates were greater in anaerobic rather than aerobic environments. This was shown both by the high correlations found between methylmercury concentration and Eh values, and, methylmercury concentration and sulphide levels in sediments. The poorer correlations that were invariably found between methylmercury contents and Eh values of sediments were probably due to the high imprecision and other problems associated with taking Eh measurements (see Chapter 9).

Third, sulphur species are very effective scavengers for methylmercury[198]. Therefore, methylmercury and sulphide levels in sediments may be expected to increase proportionally.

It is difficult to assess accurately the relative importance of the 3 factors listed above in controlling methylmercury levels in the sediment environment. However, the results of differential incubation experiments reported in Chapter 16 indicate that microbiological processes play a crucial role in determining sediment methylmercury levels.

The maximum point in the methylmercury/sulphide relationship, demonstrated by the results of the Carron and 1982 Clyde surveys, may be attributed to three factors.

First, as sediment sulphide concentrations increase, a greater proportion of the total mercury content of

sediments may be expected to be in the form of mercuric sulphide. Mercuric sulphide has been shown to produce only very low yields of methylmercury on incubation in natural sediments, both in this and other work[62]. Low rates of methylmercury formation may therefore be expected in sediments with high sulphide contents.

Second, aerobic microorganisms with the capacity to methylate mercury may be expected to function at a reduced rate, or even cease to function altogether, in environments which are high in sulphide content.

Third, as sediment sulphide concentrations increase, loss of methylmercury via dismutation through the sulphide route may be expected to become more predominant. The results of experiments reported in Chapter 19 indicate that high sulphide concentrations are necessary before significant quantities of methylmercury are lost from the sediment environment through this route.

The results of the November 1982 Carron and June 1983 Clyde surveys failed to demonstrate a relationship between the total mercury and organic carbon contents of sediments in these estuaries. High correlations between these parameters have been found in sediments of other estuaries[46, 47], and in the present work a strong relationship between the two parameters was found in sediments of the Mersey. The anomalous results of the Carron and Clyde surveys suggest that total mercury levels in sediments of these estuaries are determined primarily by local pollution inputs, i.e. high sediment total mercury levels are found in the vicinity of sewage and industrial outfalls.

Synthetic Work

The results reported in the section of the thesis entitled 'Synthetic Work' demonstrated that rates of conversion of inorganic mercury to the methyl form in the sediment environment are highly dependent upon the chemical form of

mercury present.

The results of incubation experiments reported in Chapter 17 demonstrated the importance of the chemical form of mercury in determining reactivity towards natural methylating agents. Mercuric sulphide and complexes of Hg(II) with amino acids containing sulphydryl groups, e.g. cysteine and penicillamine, were found to be unreactive towards natural methyl carbanion donors, e.g. $Me(B_{12})$ and low molecular weight compounds present in the fulvic acid fraction of sediments. Conversely, complexes of Hg(II) with amino acids containing thio ether groups, e.g. methionine and ethionine, were found to react with methyl carbanion donors. Other Hg(II) compounds were found to react with methyl carbanion donors, and the reactivity of these compounds appeared to correlate with the degree to which they dissociated in solution to produce Hg^{2+}.

Although the methylation of Hg (0) by iodomethane, a natural methylcarbonium ion donor, has been demonstrated in a pure chemical system[189], attempts to repeat this reaction in a sediment matrix were unsuccessful. An attempted methylation of Hg (0) by betaine, another natural methyl carbonium ion donor, also failed. The results of these experiments suggest the Hg (0) may only be methylated in the environment if the element is in a finely-divided or reactive form.

The results of the differential sediment incubation experiments reported in Chapter 16 demonstrated the importance of microbiological processes in converting inorganic mercury to the methyl form in the sediment environment. Much higher yields of methylmercury were obtained from the incubation of mercury compounds (with the exception of mercuric acetate) in natural rather than sterile sediments A likely explanation for these observations is that many mercury compounds are degraded in natural sediments by microbiological processes, and the products of degradation

are readily attacked by methylating agents. This proposal is based on the observation that mercury complexes, such as $Hg(cyst)_2$ and $Hg(cyst)_2Cl$, readily produce methylmercury on incubation in natural sediments, but are immune to attack by methylating agents and also fail to yield methylmercury on incubation in sterile sediments. The results of the experiments reported in Chapter 16 also show that complexes in which mercury is coordinated to chlorine produce less methylmercury than non-chlorine-containing-mercury complexes on incubation in natural sediments. It therefore appears that the degradation of mercury complexes in the sediment environment proceeds by an enzymatic process, and this process is inhibited by chlorine. This suggestion provides a possible explanation for results reported previously by Blum and Bartha[60] which demonstrated lower methylmercury production rates in estuarine regions relative to non-saline regions. The authors incubated mercuric ion in sediments containing various concentrations of chloride ion, and found that after an incubation period of 15 days , 2.3 % of the added mercuric ion was present as methylmercury in the sediment with the lowest salinity (0.1 %), compared to a methylmercury yield of 0.05 % in the sediment with the highest salinity (3 %). The requirement for an enzymatic degradation process for mercury complexes has not been appreciated in previous work. The suggestion made here is that methylation is essentially to Hg^{2+} complexed enzymatically, and not to mercury species such as $HgCl_2$, $HgCl_4^{2-}$ etc. The suggestion that the rate of decomposition of mercury complexes to Hg^{2+}-enzyme species is one of the prime factors controlling rates of methylmercury formation in the sediment environment has not been proposed in previous work.

The equivalent amounts of methylmercury produced on incubation of $Hg(CH_3COO)_2$ in natural and sterile sediments suggest that methyl migration within the $Hg(CH_3COO)_2$ molecule is the major route leading to the formation of

methylmercury in these experiments. Furthermore, the relatively high yields of methylmercury produced in these experiments suggest that formation of $Hg(CH_3COO)_2$ in the environment - e.g. by reaction of Hg(II) with acetic acid, a natural fermentation product - and subsequent intramolecular rearrangement may be a significant route leading to the formation of methylmercury in sediments.

The results of the kinetic experiments reported in Chapter 18 demonstrated that mercury was likely to be less coordinated in the sediment environment at low pH and high temperature, and thus more readily methylated. However, the effect of changes of these parameters on sediment microbiological processes is likely to be more consequential.

The results of experiments reported in Chapter 19 established conclusively the importance of the sulphide route, involving dismutation of $(MeHg)_2S$, in leading to loss of methylmercury from sediments with high sulphide contents. The yield of Me_2Hg obtained from an estuarine sediment amended with sulphide to the 4 mg g^{-1} level, but not inoculated with methylmercury, was 0.4 % of the theoretical maximum after an incubation period of 13 days. Assuming that methylmercury was lost from the sediment at a steady rate, the result suggested that the yield of $(Me)_2Hg$ after an incubation period of one year may have been as high as $\sim$11 %. The results of the experiments also suggested that yields of Me_2Hg from sediments of low sulphide content ($<$2 mg g^{-1}) may be very low or even zero. This is perhaps surprising in view of the fact that sediments with low sulphide contents (0.1 - 2.0 mg g^{-1}) contain a large stoichiometric excess of sulphide over methylmercury (ng g^{-1} range). A possible explanation is that methylmercury in the sediment environment is complexed to organic material and is thus immune to attack by sulphide. However, any methylmercury released from sediments, e.g. as a result of a biological process, may be prone to attack by sulphide, and the yields from such reactions may be expected to rise with increase in sulphide concentration. Indeed, if the

equilibrium constants for the reactions at temperatures typical of environmental conditions are low, then large quantities of sulphide may be necessary to drive the reactions and produce significant quantities of Me_2Hg. That methylmercury is released from sediments to the water column has been shown by Wright and Hamilton[199].

Conclusions

Although many factors (including mercury speciation, total mercury concentration, microbial speciation, microbial activity, temperature, Eh, pH, organic content and sulphide concentration) influence the production of methylmercury in the sediment environment, in situ levels of methylmercury in estuarine and river sediments appear to be related to sulphide concentration more than any other factor. This observation is a result of (1) direct interaction of sulphide with methylmercury and (2) inter-relationships between sulphide and the parameters listed above. The inter-relationships between sulphide and mercury speciation, total mercury levels, microbial speciation, microbial activity, Eh and organic content have been discussed in this chapter. Additionally, it may be noted that pH values and sulphide concentrations of aqueous systems are inversely related. The relationship between methylmercury and sulphide levels in the sediment environment is summarised in Fig. 61.

Different equations describing the low sulphide portion of the graph shown in Fig. 61, are derived from the results of the various surveys undertaken. These equations are listed below:-

Clyde 1982 : $[MeHg] = 1.04\,[Sulphide] + 2.91$

Clyde 1983 : $[MeHg] = 5.46\,[Sulphide] + 1.34$

Carron June 1982 : $[MeHg] = 33.24\,[Sulphide] + 8.30$

Fig. 61.

The Relationship Between Methylmercury and Sulphide Levels in the Sediment Environment

MeHg

(1) Increase in $[Hg]_{TOT}$

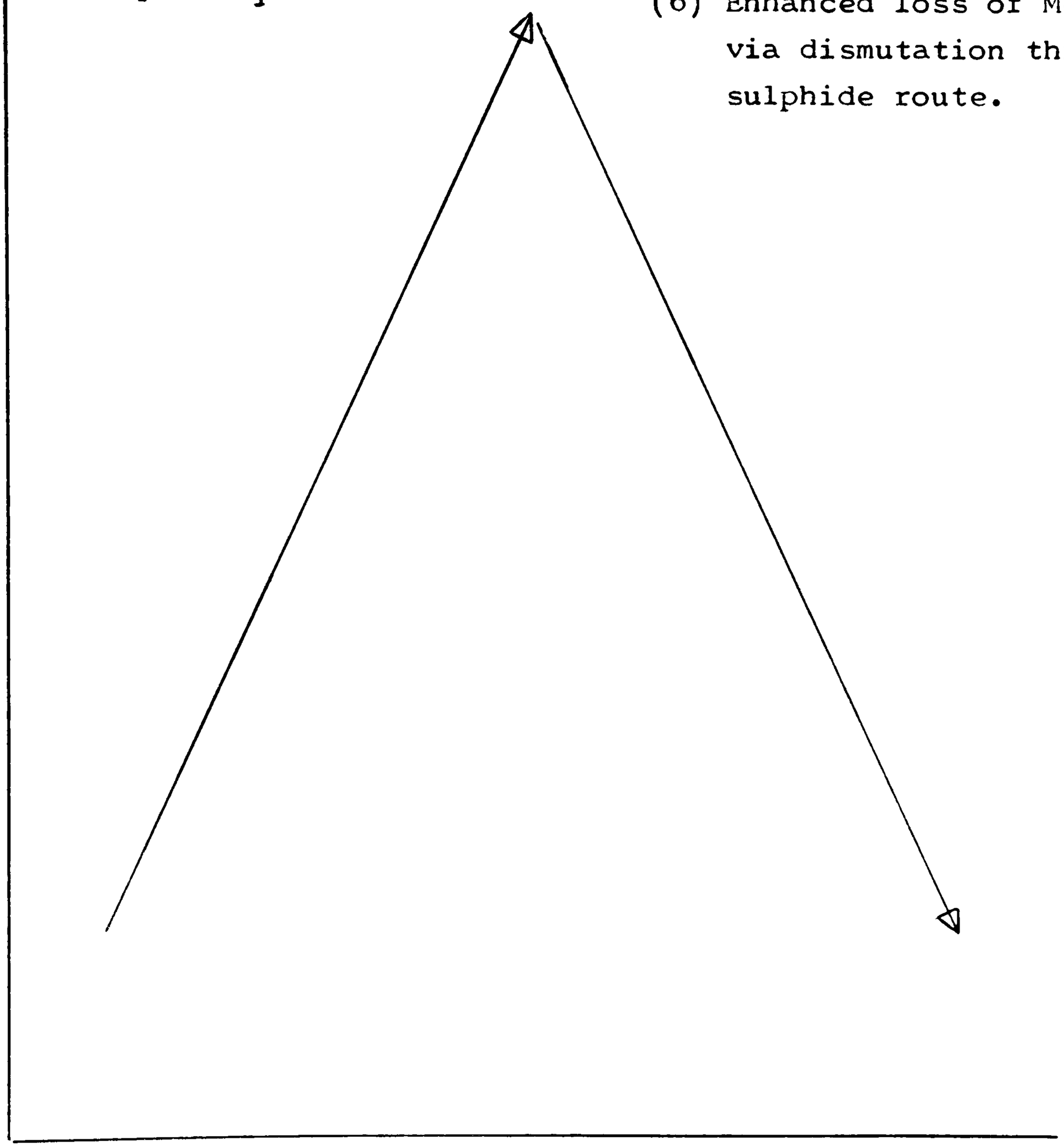
(2) Decrease in efficiency of aerobic demethylating processes.

(3) Increase in [MeHg] with increase in [sulphur species].

(4) Decrease in efficiency of aerobic methylating processes.

(5) Greater proportion of $[Hg]_{TOT}$ as HgS.

(6) Enhanced loss of MeHg via dismutation through sulphide route.



[SULPHIDE]

Carron Nov. 1982 : $[MeHg] = 21.3\,[Sulphide] + 11.08$

Plym 1982 : $[MeHg] = 1.71\,[Sulphide] + 0.75$

Teign 1982 : $[MeHg] = 0.83\,[Sulphide] + 1.13$

Dart 1982 : $[MeHg] = 1.39\,[Sulphide] + 1.20$

Mersey 1983 : $[MeHg] = 27.48\,[Sulphide] + 1.12$

units of $[MeHg]$ = ng g^{-1}, units of $[Sulphide]$ = mg g^{-1}

Differences in the methylmercury/sulphide relationship demonstrated by the above equations may be a consequence of different speciation for the inorganic mercury entering each river, different efficiences for the biotic or abiotic species responsible for methylation, or a different balance between methylation and demethylation of mercury for each location. Variations in the methylmercury/sulphide relationship in Carron and Clyde sediments demonstrated by the results of different surveys of each river, may be attributed to the collection of samples from different locations during each survey, and, perhaps, changes in pollution input to the rivers with time. Additionally, surveys of the Carron were undertaken at different times of the year when microbiological processes occuring within sediments may be expected to proceed at different rates.

Finally, the average percentage methylmercury / total mercury ratios calculated from the results of each survey may be compared; these are shown below:

Survey	$\frac{MeHg}{[Hg]_{TOT.}} \times 100$
S.W. Estuaries 1981	0.80 %
S.W. Estuaries 1982	0.82 %
Carron Nov. 1981	1.30 %
Carron June 1982	0.93 %

cont'd/...

Carron Nov. 1982	1.17 %
Clyde 1982	1.50 %
Clyde 1983	3.12 %
Mersey 1983	0.72 %

The results presented above are remarkably similar, and show that, on average, methylmercury accounts for about 1% of the total mercury present in the sediment environment. Similar results have been reported by other workers (54, 59, 102, 103, 200).

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APPENDICES

Appendix 1.

Computer Program for Calculating Least Squares Parameters

```
10 INPUT"■HOW MANY DATA POINTS";N
20 DIM X(N),Y(N)
30 LET K=0
40 FOR I=1 TO N
50 IF K=0 THEN PRINT"ENTER DATA 1 POINT PER LINE (X,Y)■"
60 LET K=K+1:J=I
70 PRINT"POINT NO.";J;"                                    ■■■■■■■■■■■■■■■■";
80 INPUT X(J),Y(J)
90 IF K<>10 AND I<>N THEN 150
100 PRINT"■■■■■■■■■■■■■■■■■■■■■■■■DO YOU NEED TO CHANGE ANY DATA"
110 GET A$:IF A$="" THEN 110
120 IF A$="N" THEN K=0:PRINT"■":GOTO 150
130 IF A$<>"Y" THEN 110
135 INPUT"■POINT NO.";J:PRINT"┐                    ":PRINT"■■■";:L=J
140 IF L>10 THEN L=L-10:GOTO 140
145 FOR M=1 TO L:PRINT"■";:NEXT M:GOTO 70
150 NEXT I
200 LET S=0:SX=0:SY=0:XY=0:X2=0:Y2=0
210 FOR I=1 TO N
220 LET SX=SX+X(I):SY=SY+Y(I)
230 LET X2=X2+X(I)*X(I):Y2=Y2+Y(I)*Y(I):S=S+X(I)*Y(I)
240 NEXT I
250 LET D=N*X2-SX*SX
260 LET M=(N*S-SX*SY)/D
270 LET C=(SY-M*SX)/N
280 LET R=(N*S-SX*SY)/SQR((N*X2-SX*SX)*(N*Y2-SY*SY))
290 LET S=SQR((Y2-M*S-C*SY)/(N-2))
300 PRINT"┐GRADIENT              =";M
310 PRINT"■INTERCEPT              =";C
320 PRINT"■STD. DEVIATION         =";S
330 PRINT"■CORR. COEFFICIENT      =";R
335 PRINT"■■IS THE PRINTER CONNECTED ?"
340 GET A$:IF A$="" THEN 340
350 IF A$="N" THEN END
360 IF A$<>"Y" THEN 340
390 OPEN 1,4:OPEN 2,4,2:OPEN3,4,1
395 PRINT#2,"AAAAAAAAAAAAAAAAAAAAAA 9999.9999
400 PRINT#1:PRINT#3,"GRADIENT              ="CHR$(29)M
410 PRINT#1:PRINT#3,"INTERCEPT             ="CHR$(29)C
420 PRINT#1:PRINT#3,"STD. DEVIATION        ="CHR$(29)S
430 PRINT#1:PRINT#3,"CORR. COEFFICIENT     ="CHR$(29)R
440 PRINT#2:PRINT#3:PRINT#3
450 PRINT#1,"   X(OBS)          Y(OBS)          Y(CAL)       DEVIATION"
460 PRINT#2,"9999.9999     9999.9999     9999.9999     9999.9999"
470 FOR I=1 TO N:PRINT#3,X(I),Y(I),M*X(I)+C,Y(I)-M*X(I)-C:NEXT
480 PRINT#2:PRINT#3:CLOSE 1:CLOSE 2:CLOSE 3:END

READY.
```

Appendix 2

Environmental Technology Letters, Vol. 3, pp. 511-520
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METHODS FOR THE ANALYSIS OF SULPHIDE IN ENVIRONMENTAL SAMPLES

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ABSTRACT

Four methods for the analysis of sulphides in environmental sediments are described and compared. Data enabling comparison between the methods is presented. The accuracy and precision for each of the methods is discussed.

INTRODUCTION

Methods for the determination of sulphide in sediments and waters can generally be grouped into four categories: iodmetric, colorimetric, electrochemically by the use of sulphide specific electrodes and spectrophotometrically by U.V. absorption of H_2S released upon acid treatment of samples.

The reduction of iodine to iodide by sulphide forms the basis of the iodometric method. To make the method specific acidification of samples under an inert atmosphere is required as a first step. The evolved H_2S is flushed into a trapping solution - e.g. zinc acetate, where the sulphide is precipitated as ZnS - which is subsequently mixed with standard iodine solution, acidifed and back titrated with thiosulphate. This method is often used to determine sulphide in natural waters and sediments, and a number of experimental procedures have been published [1]. There are a number of drawbacks to this method, e.g. it is slow, requires much glassware and extraction efficiencies are variable [2]. A variant on this method is to titrate the sample directly with iodine, but as iodine will oxidise other species present in natural waters and sediments, this approach is little more than indicative.

Colorimetric methods are commonly used to determine sulphide concentrations in non-turbid solutions and are more suitable than the iodometric method for determining low levels of sulphide (ppb range). Most published methods are based on the reaction between para-aminodimethylanaline, $FeCl_3$ and sulphide ion resulting in the formation of methylene blue ($C_{16}H_{18}N_3SCl$) [3]. This method is not suitable for the direct analysis of sediment samples as suspended material and turbidity interfere, but even with clear waters it is subject to interferences [3]. The method can be made specific for sulphide by prior treatment of the sample with acid to liberate H_2S but this incurs some of the disadvantages mentioned for the iodometric method. Other variants on this method have been published [4,5,6,7]. A major drawback of colorimetric methods is the necessity to prepare standard sulphide solutions which are unstable to atmospheric oxidation.

In recent years the use of sulphide specific electrodes has become important. Most of the sulphide electrodes now commercially available are solid state membrane electrodes employing a disc of crystalline silver sulphide [8]. The disc acts as a solid-state ion-exchange membrane specific for silver and sulphide ions and allows

these ions to impose a potential on an internal electrode contained within the main electrode body. The magnitude of the imposed potential depends upon the concentration, or more precisely, the activity, of the ions in the sample. It should be noted that these electrodes respond only to divalent sulphide ions (and Ag^+), hydrosulphide ions and H_2S produce no response. The relative ratios of divalent sulphide ion, hydrosulphide ion and H_2S in aqueous samples vary with pH and temperature, and the response of the electrode is thus governed by the pH and temperature of the sample. Also, the response of the electrode depends upon the ionic strength of the sample as the electrode responds to activity, not concentration, of ions.

Berner [9] and Whitfield [10] have used sulphide electrodes in conjunction with pH electrodes to directly measure sulphide concentrations in sediment pore waters. A drawback of this method is the necessity of preparing sulphide standard solutions to calibrate the sulphide electrode. Another electrode method has been developed by Green and Schnitker [11] who determined sulphide levels in sediments by potentiometric titration using a commercial electrode. In this method sediment samples were dispersed in a sulphide anti-oxidant buffer (SAOB - a solution of sodium hydroxide and ascorbic acid) and titrated with standard cadmium nitrate solution, the end-point being detected potentiometrically. SAOB prevents oxidation of sulphide by reducing any oxygen present and also stabilises the redox potential at a value at which sulphide oxidation is unfavourable. The high alkalinity of the SAOB also converts hydrogen sulphide and hydrosulphide ion into divalent sulphide ion which is detectable by the electrode.

Cresser [12] and Syty [13] have shown that sulphide concentrations can be determined by measurement of U.V. absorption of H_2S evolved upon acidification of aqueous samples. The method makes use of an atomic absorption spectrometer modified for cold vapour analysis. H_2S evolved from the sample is flushed into a flow-through cell and the absorbance from a deuterium lamp is recorded at 200 nm; the sulphide concentration is determined from a calibration graph. Sediments cannot be directly analysed by this method as the difference in matrix composition of the samples and calibration standards may result in different rates of release of H_2S. However, it has been suggested that water-soluble sulphide in sediments be extracted into SAOB solution which may then be analysed in the normal way after centrifuging to remove suspended solid matter. Sediments analysed in this way gave higher results than those obtained by standard ion-selective electrode techniques [12]. This could be due to the presence of colloidal iron sulphide and other interferents in the SAOB extract.

Other methods have been developed but they are, by comparison, little used.

Our interest in sulphide levels in the environment stems from their role in acting as a "sink" for heavy metals and in their more recently discovered role in mobilising mercury as dimethyl mercury [14]. We have previously pointed out the importance of the interaction of CH_3Hg and S^{2-} to produce volatile $(CH_3)_2Hg$ in the environment, viz:-

$$2CH_3Hg^+ + S^{2-} \rightarrow (CH_3Hg)_2S \rightarrow (CH_3)_2Hg + HgS \quad [14]$$

There are indications that this reaction becomes dominant at levels of sulphide > 5 mg g^{-1} and hence we have been interested in measuring sulphide concs. in natural sediments. Below $[S^{2-}]$ = 5 mg g^{-1} the conc. of MeHg appears to be approximately proportional to $[S^{2-}]$ [15,16].

In view of the lack of stability of sulphide in sediments exposed to air during sampling and the precautions necessary to preserve sediment samples, and also because the analytical techniques described above measure different sulphide species, it was felt important to develop a comparison between them in order to extrapolate between different methods and measurements, and to make proper comparison with previously published work.

EXPERIMENTAL

Digested sewage sludge was obtained from Wanlip Sewage Treatment Plant, Leicestershire (Severn Trent Water Authority, U.K.). The sludge was allowed to settle and the supernatent liquid decanted from it. The sulphide content of replicate samples of the remaining solids was determined by four methods: (1) direct iodometric titration, (2) volatilisation of H_2S followed by iodometric titration (indirect iodometric), (3) potentiometric titration and (4) gas-phase molecular absorption spectroscopy (G.M.A.S.).

Individual samples were taken from a homogenised 1 Kg sample of sewage sludge solids. The samples were weighed quickly to avoid changes in sulphide levels due to oxidation and microbiological activity. Samples for analysis by methods (1) and (2) were analysed immediately after being weighed; samples for analysis by methods (3) and (4) were preserved in SAOB solution and analysed as soon as possible to avoid any differences caused by deterioration of the samples.

The dry weight of the sewage sludge solids was determined by drying 10g of sample at 110°C to constant weight. Water content ranged from 54% to 56%.

REAGENTS

Iodine (A.R.)	- 0.005 mol dm^{-3} aqueous
Sodium thiosulphate (A.R.)	- 0.01 mol dm^{-3} aqueous
Zinc acetate	- 2 mol dm^{-3} aqueous
Sulphuric acid	- 50% v/v
Cadmium nitrate (A.R.)	- 0.001 mol dm^{-3} aqueous
Hydrochloric acid	- 50% v/v

SAOB: Prepared by dissolving 560g of potassium hydroxide and 17.6g of ascorbic acid in 1 dm^{-3} of deoxygenated distilled water and kept anaerobic.

(1) Direct Iodometric

Up to 1g of wet sample was weighed into a 10 cm^3 centrifuge tube to which 10 cm^3 of standard iodine solution was then added. The tube was stoppered and shaken for two minutes. The mixture was left for 1 hour and centrifuged. After centrifuging, 5 cm^3 of solution containing excess iodine was removed and titrated with standard thiosulphate solution.

Five portions of sewage sludge were analysed as described; the results are presented in Table 1.

(2) Indirect Iodometric

The appartus used for the evolution and trapping of H_2S is illustrated in Fig.1. About 5g of wet sample were weighed into the digestion vessel, 50 cm^3 of zinc acetate solution was introduced into the conical flask and the apparatus connected as shown. Nitrogen was passed through the vessel to displace air, and the sample then acidified by injecting 20 cm^3 of sulphuric acid through the injection port. The digestion vessel was heated to 100°C by the hot plate and a flow of nitrogen maintained through the system for 1 hour, until all of the evolved H_2S had been carried over. The bubbler tube was then removed from the conical flask and 20 cm^3 of iodine solution, followed by 5 cm^3 of concentrated hydrochloric acid, were added to the flask. The flask was stoppered and shaken for about 10 seconds and the excess iodine was then immediately titrated with standard thiosulphate solution.

Five portions of sewage sludge solids, identical with those analysed by Method 1, were analysed by the above method and the results are presented in Table 1.

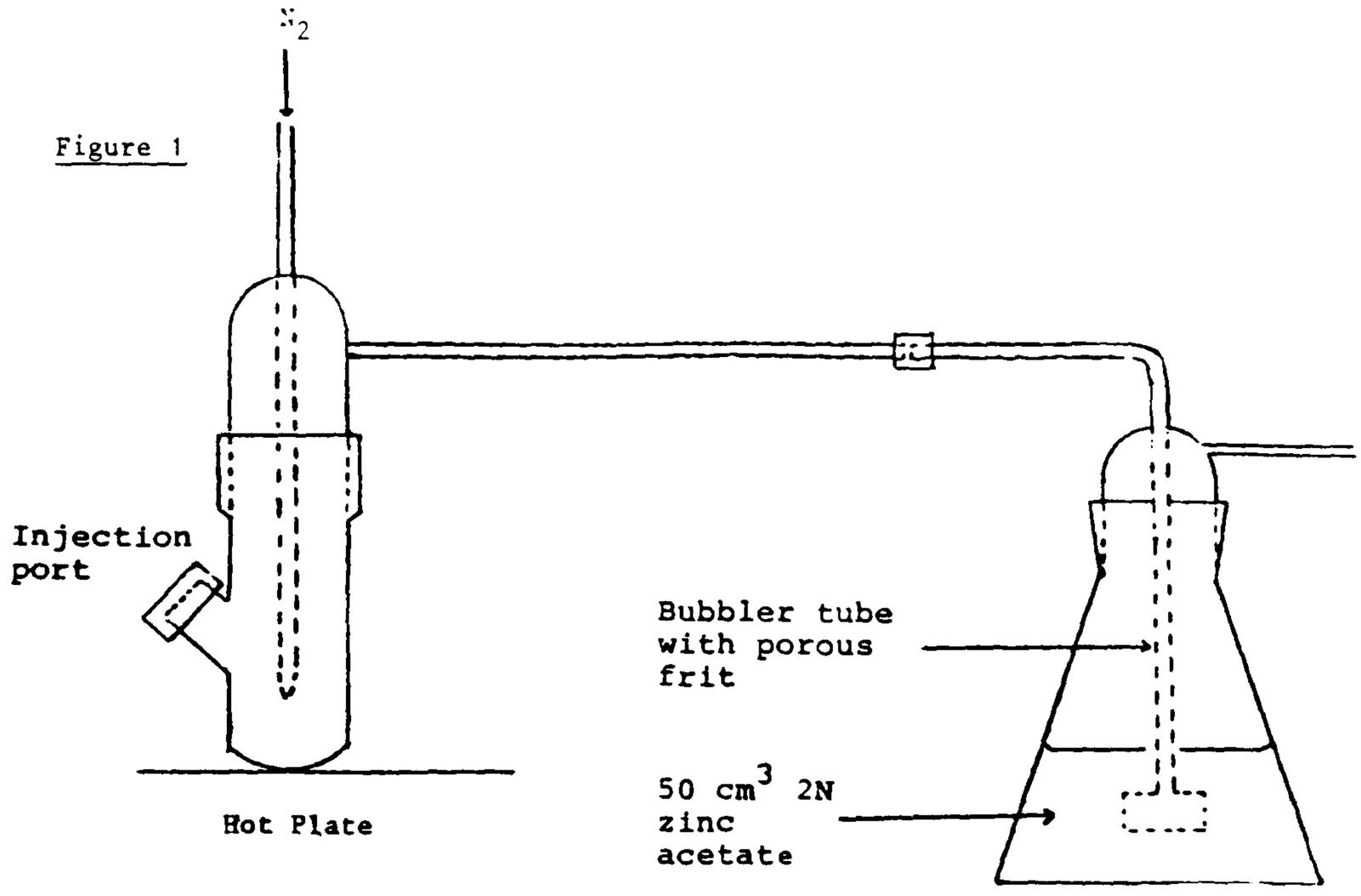

Figure 1

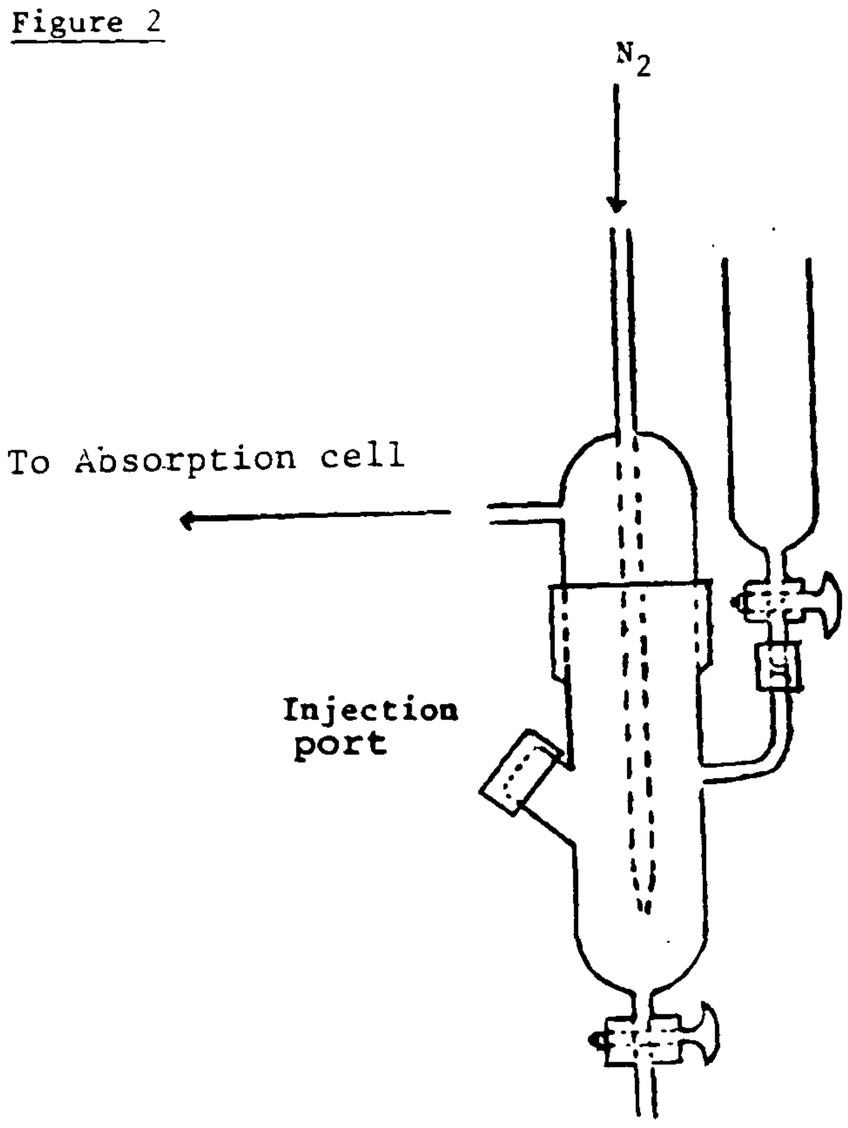

Figure 2

(3) Potentiometric Titration

An Orion Research Inc. Model 94-16 sulphide electrode was used in conjunction with an Orion Model 90-02 double-junction reference electrode. The outer chamber of the reference electrode was filled with 10% KNO_3 solution. A digital voltmeter was used to monitor the potential of the electrodes.

Between 0.5 and 1g of wet sample was weighed into a 250 cm^3 beaker. To the beaker were then added 50 cm^3 of SAOB and 50 cm^3 of distilled water, and the sample was dispersed in the SAOB solution by vigorous stirring. The indicating and reference electrodes were immersed in the solution which was then titrated with $Cd(NO_3)_2$ solution. The solution was continuously stirred throughout the course of the titration by a magnetic follower.

The change in electrode potential with addition of titrant to form CdS was recorded and a titration curve plotted; the end-point was found from the point of inflection in the curve. No sulphide was detected in a SAOB solution blank. Five portions of sewage sludge solids, identical with those analysed by Methods 1 and 2, were analysed by the above method and the results are presented in Table 1.

(4) Gas-Phase Molecular Absorption Spectrometry (GMAS)

An I.L. Inc. Model 151 atomic absorption spectrometer modified for non-flame cold vapour analysis was used to make absorption measurements of evolved hydrogen sulphide. The gas was led into a 10 cm long quartz-windowed flow-through absorption tube situated in the light path of a deuterium hollow cathode lamp. Absorption measurements were made at 200 nm and were recorded as peak heights on a chart recorder. The digestion vessel used for the evolution of H_2S is essentially that described by Syty and is illustrated in Fig.2. Nitrogen was used to sweep H_2S out of the vessel and into the absorption cell.

A calibration graph was prepared by injecting 1 cm^3 aliquots of standard solutions (made up in 25% SAOB solution) into 20 cm^3 of 50% HCl. Five replicate injections for each of the sulphide concentrations were made; the results are presented below:

Concentration of Sulphide (mg $dm^{-3}S^{-2}$)	Average Absorption Peak Height (mm)	Standard Deviation (mm)	Coefficient of Variation (%)
94.0	78	3.59	3.9
70.5	57	2.39	4.2
47.0	39	1.60	4.1
23.5	17	1.51	8.9
9.4	8	1.10	13.8

The H_2S gas measured was generated after weighing approximately 15g of wet sample into a 250 cm^3 beaker. SAOB (25 cm^3), followed by distilled water (75 cm^3), was added to the beaker and the sample dispersed in the SAOB solution by vigorous stirring. A portion of the resultant suspension was centrifuged, and from a 1 cm^3 aliquot of the supernatant liquid H_2S was evolved for analysis by injection into 20 cm^3 of 50% HCl.

Five portions of sewage sludge solids, identical to those analysed by the other methods, were analysed as described and the results are presented in Table 1.

Experiments were also performed to assess the recovery of added sulphide from sewage sludge; quantitative recoveries (> 90%) were obtained for all four methods.

RESULTS AND DISCUSSION

The average sulphide concentration of the homogenised sewage sludge solids obtained by the four methods, together with values for the standard deviation and coefficient of variation for replicates within the four methods, are presented in Table 1.

TABLE 1: RESULTS OF ANALYTICAL METHODS - WANLIP SAMPLES

METHOD	[SULPHIDE] ($mg\ g^{-1}$)*	STD. DEV. ($mg\ g^{-1}$)	COEFF. VAR.
1. DIRECT IODOMETRIC	1.97	0.10	5.0%
2. INDIRECT IODOMETRIC	0.83	0.08	9.2%
3. POTENTIOMETRIC TITRATION	0.46	0.01	2.5%
4. G.M.A.S.	0.72	0.09	12.1%
	*Dry weight		

Method 2 determines both acid-soluble and water-soluble sulphide [15]; Methods 3 and 4 theoretically determine water-soluble sulphide only, whereas Method 1 has been shown to determine water-soluble sulphide and a part of the acid-soluble fraction [15]. On this basis Method 2 would be expected to register the highest sulphide levels. However, the highest levels of sulphide were in fact found from Method 1. Since good sulphide recoveries were obtained from spiked sediments by Method 2 (92%), it is clear that the direct iodometric method is subject to considerable interference; sulphite and thiosulphate are known to be likely interferents for this method [17] and these are present in sediments and sludges.

Methods 3 and 4 would be expected to register similar sulphide levels. However, like others [12] we obtained higher results by the GMAS method, suggesting that it also is subject to interference. Possible interferents for this method might include sulphite, nitrite, sulphide complexes with organic matter and Fe(II), and colloidal iron sulphide which may not be completely removed in the centrifugation step of the analysis [12,13].

Variation in the precision of the four methods was also found, the most precise results being obtained by Method 3.

This series of experiments was repeated using homogenised sediments collected from the River Carron (Lothian, Scotland), Cropston Reservoir (Leicestershire) and separate samples of sewage sludge obtained from Wanlip (Leicestershire). The results are presented in Tables 2-4.

TABLE 2: CROPSTON SEDIMENTS

METHOD	[SULPHIDE] ($mg\ g^{-1}$)	STD. DEV. ($mg\ g^{-1}$)	COEFF.VAR.
1. DIRECT IODOMETRIC	1.73	0.07	4.4%
2. INDIRECT IODOMETRIC	1.11	0.10	9.3%
3. POTENTIOMETRIC TITRATION	0.83	0.02	2.5%
4. G.M.A.S.	0.91	0.08	9.2%

TABLE 3: CARRON SEDIMENTS

METHOD	[SULPHIDE] (mg g^{-1})	STD.DEV. (mg g^{-1})	COEFF. VAR.
1. DIRECT IODOMETRIC	2.69	0.14	5.1%
2. INDIRECT IODOMETRIC	1.43	0.14	9.8%
3. POTENTIOMETRIC TITRATION	1.24	0.03	2.7%
4. G.M.A.S.	1.12	0.10	9.1%

TABLE 4: WANLIP SAMPLES (BATCH 2)

METHOD	[SULPHIDE] (mg g^{-1})	STD.DEV. (mg g^{-1})	COEFF. VAR.
1. DIRECT IODOMETRIC	3.63	0.25	6.9%
2. INDIRECT IODOMETRIC	1.42	0.14	9.8%
3. POTENTIOMETRIC TITRATION	0.93	0.05	4.8%
4. G.M.A.S.	1.40	0.14	9.8%

The following conclusions can be drawn from the results presented in Tables 1-4:

1. The lower ratios of results from Methods 2:3 obtained with non-sewage sediments indicates that there is a greater proportion of water-soluble sulphide in non-sewage sediments compared with sewage sludge solids. Ratios of results from Methods 2:3 from Tables 1-4 are 1.8, 1.3, 1.2 and 1.5 respectively.

2. Methods 3 and 4 registered similar levels of sulphide in the analysis of Carron and Cropston sediments, whereas substantially higher levels of sulphide were obtained by Method 4 in the analysis of both batches of sewage sludge. This suggests that the interferents for the GMAS method are present in higher concentrations in sewage sludge than in sediments. Ratios of Methods 4:3 from Tables 1-4 are 1.6, 1.1, 1.1 and 1.5 respectively.

3. Method 1 compared to Method 3 also has lower ratios for non-sewage sediments for similar reasons to (1) above. Ratios of results from methods 1:3 are 4.3, 2.1, 2.2 and 3.9 respectively.

4. The level of precision of a given method remained closely constant over different types of sample, Method 3 being consistently the most precise average coefficient of variation = 3.1%).

Previous workers [15,16] from our laboratory have determined sulphide concentrations in sediments using the direct iodometric method. However, this present study has shown that the method is prone to interference, and we now recommend the potentiometric titration method which has the advantages of being precise, relatively immune to interferents and suitable for the analysis of large numbers of samples. Although the direct Iodometric method overestimates true sulphide concentrations, the ratios between this and the preferred Method 3 appears to lie close to 2:1 for natural environmental samples and a normal conversion therefore appears valid between the

two methods. For sewage sediments Method 1 appears to over-estimate true sulphide concentrations by a factor of four.

Finally, a group of Carron River sediments containing varying amounts of sulphide were analysed by both the direct iodometric and the potentiometric titration methods (1 and 3) to determine if a general relationship exists between the results obtained by the two methods. Twenty-two sediment samples were analysed; a plot of the data is shown in Figure 3. A least squares analysis of this data produced the following equation for the straight line: $y = 1.58x + 0.73$ with a linear correlation coefficient of 0.92. Figure 3 suggests that an approximately linear relationship exists between the two methods and that the data may be used to correlate results derived from each method for a given sediment. This in fact is now being carried out in the analysis of our previous work.

CONCLUSIONS

A detailed comparative study of four methods used for the analysis of environmental sulphide has been carried out. The results suggest that the relationship between the most convenient (Method 1) and the most accurate (Method 3) methods is approximately linear, and that for a given sediment matrix either method may be used and Method 1 may be statistically extrapolated to produce true sulphide levels. The relationship between sediment types and results produced from the various methods of analysis are being studied further for a wider variety of sediments. Present results do strongly bring out the different behaviours of sewage sludges compared to natural sediments between the four methods of analysis.

ACKNOWLEDGEMENTS

We thank the Severn Trent Water Authority (Mr. K. Foster) for assistance in sample collection from Cropston Reservoir and Wanlip. We also thank Dr. Tom Leatherland (Forth River Purification Board) and Dr. David Taylor (ICI Ltd., Brixham Laboratory) for help in the collection and analysis of samples. Particularly, thanks are expressed to ICI at Brixham for provision of laboratory space.

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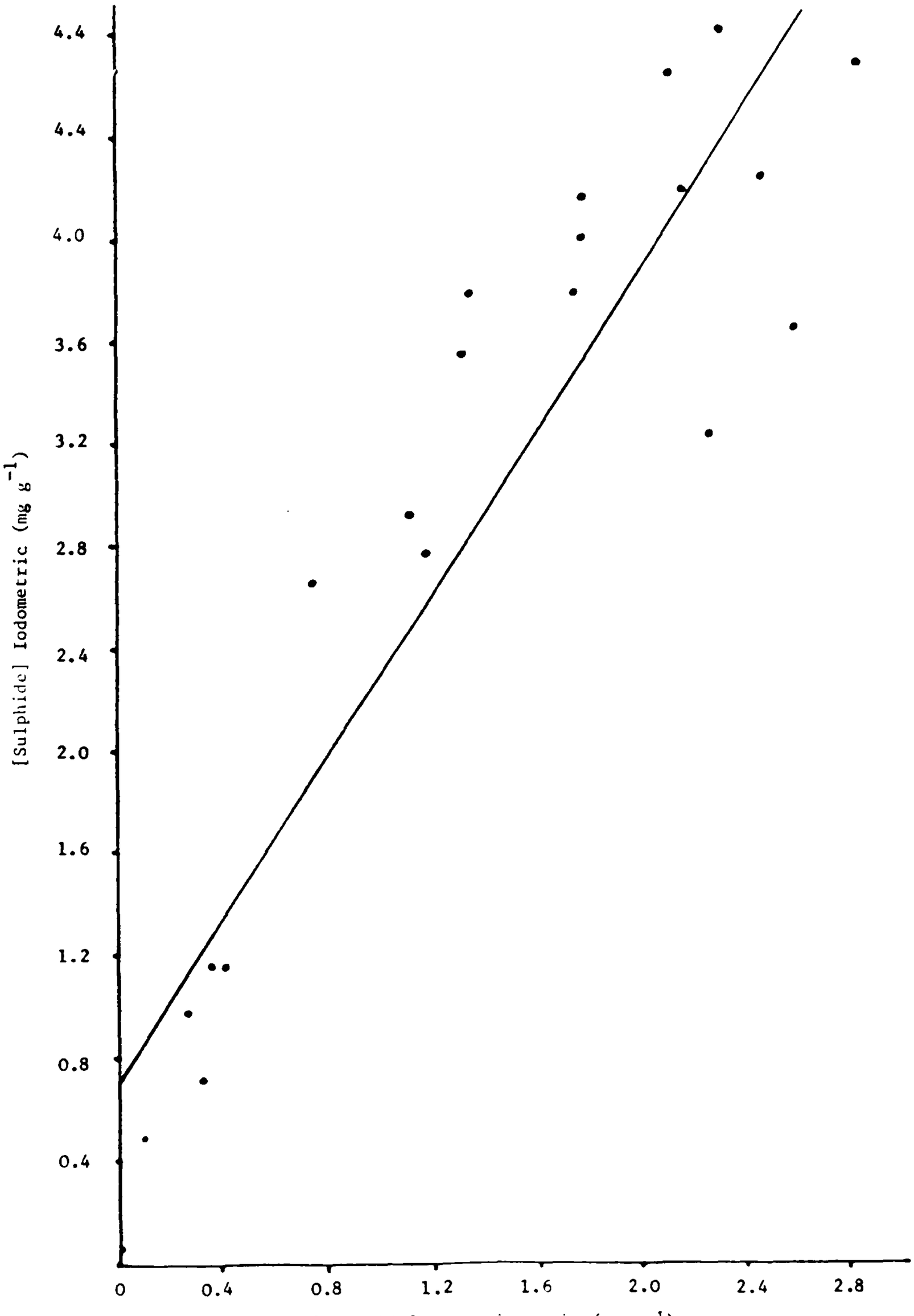
Figure 3: [Sulphide] Direct Iodometric vs. [Sulphide] Potentiometric (River Carron Sediments)



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Appendix 3.

REPORTS

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Printed in Great Britain

0025-326X/83 $3.00 + 0.00

Total Mercury, Methyl Mercury and Sulphide in River Carron Sediments

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Total mercury, methyl mercury and sulphide contents of River Carron sediments (Lothian, Scotland) have been determined. Total mercury concentrations are comparable to other mercury polluted estuaries in the UK, but the methyl mercury values for low-sulphide Carron sediments are generally higher. It has been found that methyl mercury levels are initially in direct proportion to the sulphide concentrations of the sediments but beyond sulphide concentrations of 1.8 mg g^{-1} the methyl mercury levels decline sharply.

The River Carron flows through the Lothian region, Scotland, and joins the Firth of Forth at Grangemouth (Fig. 1). It is a polluted river having suffered from urban and industrial waste emission, including several sewage discharges and a point source for mercury input from the effluent of a chemical complex. There is also a discharge from a paper mill. The average flow rate of the Carron varies from 11.6 to 1.1 $m^3 s^{-1}$ during the year, the rate being at a maximum in November and at a minimum in August (FRPB, 1981).

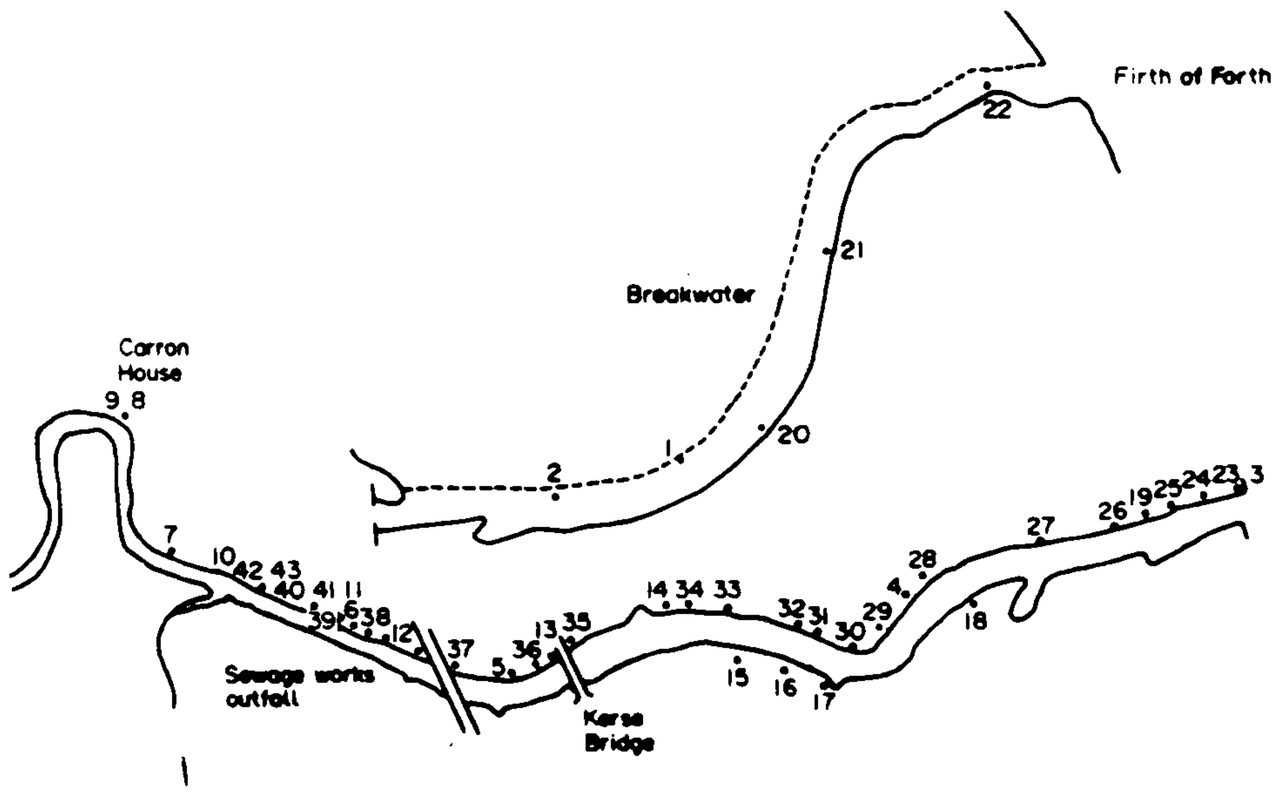

Fig. 1 Sampling stations, River Carron, Scotland.

Sampling and Analysis

Sediment samples were collected from Carron intertidal locations at low water (Fig. 1). The samples were obtained from the top 3–5 cm of the sediment and placed directly into polythene bottles. These were filled completely with sediment and closed by gas-tight caps to prevent exposure to air. These samples were kept at ambient temperature prior to analysis for methyl mercury and sulphide content (within a few hours of collection). For comparison some samples were frozen with solid carbon dioxide on collection and stored frozen before analysis (up to one week later). These sediments were frozen in order to assess any changes in methyl mercury and sulphide levels during transport and storage. Total mercury levels are not affected by storage temperatures up to 15°C (Craig & Morton, 1976).

The total mercury content of the sediments was determined by cold vapour atomic absorption (CVAA) spectrometry (Craig & Morton, 1976). Methyl mercury levels were determined by gas chromatography (GC) using an electron capture detector (Bartlett *et al.*, 1977, 1978). Sulphide levels were determined by potentiometric titration with cadmium (II) nitrate solution employing a commercial ion selective electrode (Craig & Moreton, 1982). Redox potentials (Eh) were measured with a platinum–calomel electrode system. (Orion Model 94–16 sulphide electrode and Model 90–02 double junction reference electrodes were used for the sulphide measurements; Orion Model 96-78 was used to measure Eh values.) Correlations were assessed by calculation of linear correlation coefficients (r).

Results and Discussion

Three surveys of the surface intertidal sediment zone of the Carron (Fig. 1) were undertaken during the period November 1981, July 1982 and November 1982. The results of these surveys are presented in Table 1. The most interesting feature of the data is the relationship beween methyl mercury and sulphide levels.

A composite plot of ambient methyl mercury and sulphide concentrations obtained from the three surveys (Fig. 2) shows that methyl mercury concentrations rise initially with increase in sulphide concentration, but that after a concentration of about 1.8 mg g^{-1} of sulphide is exceeded, methyl mercury concentrations decay with further increase in sulphide concentrations. A poor correlation between methyl mercury and both total mercury and organic carbon content was generally found in these locations and sulphide content seems to be the controlling factor for methyl mercury. This general pattern was shown in all three surveys and was particularly evident in the more extensive survey of November 1982. The results of the July 1982 survey demonstrate a linear relationship between methyl mercury and sulphide levels up to a sulphide concentration of 1.6 mg g^{-1} ($r=0.84$). For this survey, only one sample was found to contain a sufficiently high sulphide concentration (2.44 mg g^{-1}) to present evidence of a maximum point in the graph. A poor linear correlation coefficient between methyl mercury and total mercury levels was also found in July ($r=0.46$, significant at 10% level only).

In the November 1982 survey a linear relationship between methyl mercury and lower sulphide concentrations

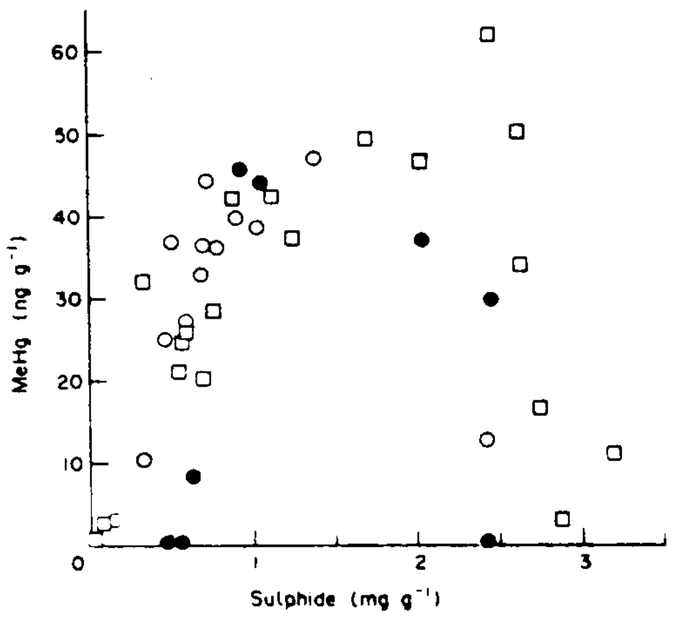

Fig. 2 The relationship between methyl mercury and sulphide content for the River Carron sediments. ● November 1981; ○ June 1982; □ November 1982.

TABLE 1

Mercury, methyl mercury and sulphide – River Carron, Lothian, Scotland, 1981–82

A. November 1981 survey

Sample No.	Sulphide* (mg g^{-1})	MeHg* (ng g^{-1})	[Hg]TOT* ($\mu g\ g^{-1}$)
1	0.48	<0.5	1.09
2	2.05	36.9	3.13
3	0.56	<0.5	0.05
4	2.44	2.9	2.51
5	0.63	8.4	1.06
6	2.49	29.8	2.45
7	1.07	44.1	2.34
8	0.94	45.6	1.73

B. July 1982 survey

Sample No.	Sulphide* (mg g^{-1})	MeHg* (ng g^{-1})	[Hg]TOT* ($\mu g\ g^{-1}$)
9	0.78	36.1	3.14
10	0.73	32.9	3.60
11	0.73	44.2	3.13
12	1.40	46.9	3.25
13	0.60	27.7	3.52
14	0.72	36.1	3.33
15	0.92	39.7	2.86
16	0.47	25.2	3.91
17	0.51	36.9	3.42
18	1.05	38.3	3.48
19	0.70	32.9	3.37
20	2.44	12.9	3.39
21	0.34	10.3	2.85
22	0.15	3.1	1.90

C. November 1982 survey

Sample No.	Eh (mv)	Sulphide* (mg g^{-1})	MeHg* (ng g^{-1})	[Hg]TOT* ($\mu g\ g^{-1}$)
23	+ 60	0.08	2.8	1.11
24	− 80	0.60	26.1	2.85
25	−110	0.64	26.9	3.95
26	−110	0.69	18.9	3.70
27	− 80	0.58	24.6	1.00
28	0	0.90	42.1	1.99
29	− 25	0.77	28.5	2.63
30	−140	1.14	42.2	2.80
31	− 70	0.55	21.0	1.53
32	− 5	0.34	31.9	2.57
33	−210	1.71	49.0	2.65
34	−200	2.49	62.0	3.84
35	−200	5.56	18.1	2.62
36	−360	2.78	16.7	2.65
37	−140	2.90	3.3	3.49
38	−100	1.26	37.3	2.51
39	− 60	2.05	6.4	2.60
40	+ 40	<0.01	0.7	0.04
41	−250	3.21	11.3	2.46
42	− 60	2.65	50.2	2.30
43	−120	2.64	34.0	2.49

*Expressed as dry weight of sediment.

was again observed (on data points for sulphide levels below 2.6 mg g^{-1} sulphide, $r = 0.90$). At sulphide concentrations above 2.6 mg g^{-1}, a decrease in methyl mercury levels was seen. Methyl mercury and total mercury levels again were found to correlate less well than methyl mercury and sulphide concentrations when the sulphide concentrations were low. The linear correlation coefficient for the methyl mercury and total mercury levels of those sediments containing less than 2.6 mg g^{-1} sulphide is 0.60; for methyl mercury and sulphide it is 0.90.

The composite Fig. 2 shows the general relationship found between methyl mercury and sulphide concentrations for this location. A similar pattern based on fewer data points was found from sediments taken from the River Clyde, Scotland (Bartlett *et al.*, 1978; Bartlett & Craig, 1981). The maximum point for the previous Clyde data was found to occur at a sulphide concentration of approximately 2.8 mg g^{-1}, whereas the corresponding point in Fig. 2 occurs at a lower sulphide concentration of between 1.8 and 2.0 mg g^{-1}. The iodometric method of sulphide determination employed in the previous analyses of the Clyde sediments detects in addition to hydrogen sulphide and water soluble inorganic sulphide, the non-relevant species thiosulphate and sulphite which also may be present in sediments (Rozanov *et al.*, 1971), and will therefore produce inflated values for sulphide levels. For this reason a specific potentiometric method was developed (Craig & Moreton, 1982). For most sediments there is a linear relationship between the specific sulphide levels in sediments determined by the potentiometric method and those recorded by the iodometric method. This conversion has been applied in determining the maximum point of 2.8 mg g^{-1} sulphide for the Clyde data (Bartlett & Craig, 1981).

Correlations between methyl mercury and organic carbon content of the sediments are generally less than between methyl mercury and sulphide levels below 1.8 mg g^{-1}. For November 1982 the correlation coefficient is 0.58, for example.

Conclusions

These results seem to confirm in detail a proposal made previously that methyl mercury levels are controlled more by the sulphide content in sediments than by factors such as total mercury levels or organic content. The previous suggestion, that methyl mercury levels in River Clyde sediments initially are directly proportional to sulphide content but then decline sharply above a certain sulphide level, was made with limited data (10 data station points). The work suggests that this relationship may be general for sediment locations having mercury and varying sulphide contents. The origin of the sharp decline in methyl mercury levels may arise from the conversion of the monomethyl species to volatile hydrophobic dimethyl mercury by disproportionation.

$$2CH_3Hg^+ + S^{2-} \rightarrow (CH_3Hg)_2S \rightarrow (CH_3)_2Hg + HgS.$$

The dimethylmercury produced is lost by diffusion through the aqueous layer and by transport in the atmosphere and this mechanism may therefore be a general component of an overall cycling process for mercury in the biosphere. The abiotic chemistry of the reaction has been demonstrated previously (Craig & Bartlett, 1978) and its feasibility in a model sediment environment has also been shown (Bartlett, 1979).

The present results from the Carron point towards the generality of a loss of methyl mercury from sediments where methyl mercury levels would otherwise be expected to be substantial and that the factor most directly involved in this loss is high sulphide concentrations contiguous to complexed mercury in the sediments.

These results also demonstrate for the first time mercury and methyl mercury levels in the Carron (Table 1). Few details for methyl mercury are yet available for UK river or estuary sediments and the present levels may be compared

to values found in sediments from the River Mersey, England. The Carron range for methyl mercury is < 0.5 to 62 ng g^{-1} while the Mersey range is < 0.5 to 43.3 ng g^{-1}. The Carron range for total mercury is < 0.05 to 3.95 μg g^{-1} and that for the Mersey is < 0.05 to 4.83 μg g^{-1}. Although total mercury concentrations for both rivers are similar, methyl mercury levels for the Carron (in those sediments which do not have high sulphide concentrations) are generally somewhat higher. This may be a consequence of different speciation for the inorganic mercury entering each river, to different efficiencies for the biotic or abiotic species responsible for methylation, or to a different balance in the equilibrium between methylation and demethylation of mercury for each location.

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Appendix 4

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METHYLATION OF MERCURY, TIN AND LEAD IN AQUEOUS AND SEDIMENT ENVIRONMENTS.

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ABSTRACT

Experiments are described on the incubation of inorganic tin, lead and mercury compounds with electrophilic methylating agents (e.g. iodomethane, betaine). Experimental conditions include aqueous media, ambient tenperature and excess of methylating agent during incubation. Identification and yields of the methyl metal compounds formed in these reactions are reported. A trideuteromethyl tin compound has been incubated in a natural sediment in order to determine the proportion of tetramethyl tin formed by disproportionation compared to biomethylation. Some experiments with methyl cobalamin (CH_3CoB_{12}) are also described, and the role of sulphide ligands present in sediments in the conversion of mono-to dimethyl mercury is discussed.

INTRODUCTION

For a number of years we have been interested in the methylation mechanism for heavy metal elements, principally mercury, tin and lead, under environmental or model environmental conditions. Recently we have investigated the reaction of natural methyl carbonium ion doners (e.g. iodomethane, betaine) with tin and lead as metals capable of undergoing oxidative addition to the tetravalent state (which is the valence state of the reported environmental methyl derivalives). For lead in particular oxidative addition of methyl carbonium ion (CH_3^+) would appear to be a more satisfactory mechanistic route to methyl lead compounds in the environment than carbanion attack on lead(II) or lead (0) with methyl cobalamin (CH_3CoB_{12}) as methyl doner. Numerous groups have reported lack of success in the reaction of CH_3CoB_{12} with inorganic lead compounds (ref.1). Carbonium ion attack in an oxidative addition would utilize the lone pair of electrons present in lead (II) or lead (0). Numerous examples are now known of methyl lead or tin compounds apparently generated in the natural aqueous environment (ref. 2,3,4). In an attempt to uncover the mechanisms of these reactions we have studied the reactions of various methyl carbonium ion doners (e.g. CH_3I, $(CH_3)_3^+NCH_2COO^-.H_2O$, $(CH_3)_3SI$, $(CH_3)_2S$ with mercury, tin and lead compounds.

We have also been interested in the proportion (if any) of the methylation of trimethyl tin and - lead complexes to the tetramethyl derivatives that is due to authentic environmental methylation rather than disproportionation viz:-

*To present paper.

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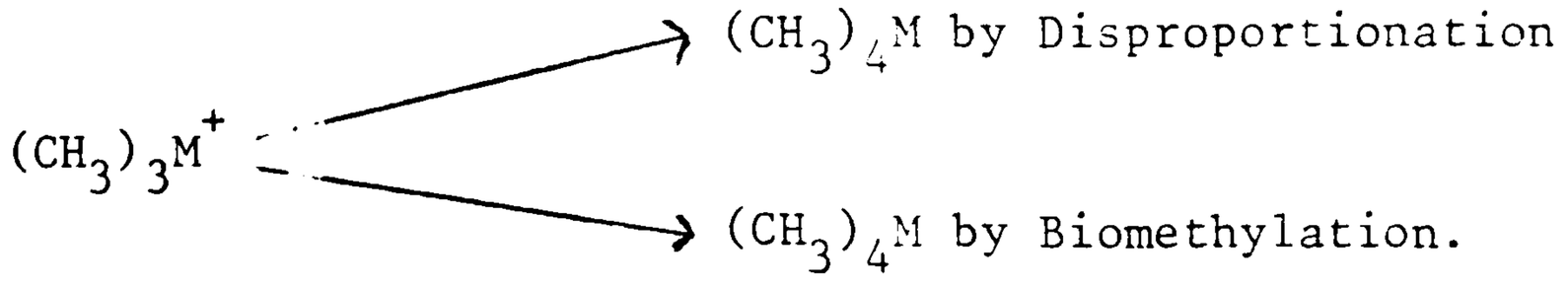

There are numerous well-established disproportionation routes including those catalysed by sulphide ligands or by solid surface catalysis (ref.5). The conversion of monomethyl - to dimethyl mercury mediated by sulphide ions is well understood (ref.5), and that for the analogous conversion of trimethyl tin and - lead has been established recently (ref.6). In view of the obvious importance of establishing whether or not a parallel natural methylation involving an environmental methyl doner (i.e. biomethylation) takes place, we have studied the incubation of trideuteromethyl tin chloride in a natural sediment in order to establish if any tetramethyl tin containing CH_3 groups is produced. Previous work has suggested that biomethylation could be occurring in this case (ref.7).

The importance of the role of sulphide in converting mono- to dimethyl mercury is shown by recent investigations of methyl mercury levels in sediments from the River Carron estuary in Scotland (ref.8). We also describe results from the reactions of CH_3CoB_{12} and other methylating agents with various mercury, lead and tin sulphur - ligand complexes which model the coordination chemistry of these metals in the natural sediment environment. In such an environment coordination of the metal may occur by complex sulphur-containing molecules and we have synthesized various metal-amino acid compounds to model methylation in the environment.

EXPERIMENTAL

CH_3I or $(CH_3)_3^+NCH_2COO^-.H_2O$, betaine (16 m mole) was incubated in aqueous solution or suspension with Pb^o, Sn^o, $SnCl_2$, Sn $[(COO)_2]$, SnS, Na_2SnO_3, $Pb(NO_3)_2$, $Pb(CH_3COO)_2$ and other metal-sulphur ligand complexes (see Table) in 50 cm^3 McCartney bottles containing 30 cm^3 H_2O. Evolution of volatile methyl metal products was tested for by withdrawal of 1 cm^3 head space aliquots and injection to g.c. (Pye Unicam 104; F.I.D. detector; column length 1.8 m; diameter 6 mm; column temperature for $(CH_3)_4Sn$ 50°, $(CH_3)_4Pb$ 80°; stationary phase 10% SP2100). Partially methylated, non volatile products (e.g. $(CH_3)_3Sn^+$) were detected by hydride generation as follows:- after uncapping and purging with N_2 (1 hour) to remove excess CH_3I (not done for betaine), 1 cm^3 of pH 7.0 buffer (Gomori's tris) was added and the flask recapped. 1.5% $NaBH_4$ reagent in water (1cm^3) was injected through a seal and the flask shaken for 2 minutes prior to g.c. analysis as described previously for volatile products now present in the head space (e.g. $(CH_3)_3SnH$). Gas chromatography coupled to mass spectroscopy was used to confirm the identities of the methyl metal products (VG micromass 16 F system).

Incubation of CH_3I with Hg^o was described many years ago with the product being CH_3HgI (ref.9). We have recently carried the incubation out in a sediment medium as follows: - Hg^o (0.025 m mole) was added to a natural sediment (50 g) in a round-bottom flask (250 cm^3) and CH_3I (0.35 m mole) was added by injection. (This gave a sediment 100 $\mu g\ g^{-1}$ in Hg^o and 1000 $\mu g\ g^{-1}$ in CH_3I). Blank

experiments without CH_3I were also carried out in order to assess the importance of CH_3I methylation by oxidative addition compared to sediment methylation including unknown methyl doners (possibly CH_3CoB_{12}). Incubation was for 7 days.

To compare natural methylation with disproportionation, $(CD_3)_3$ SnCl was synthesised and incubated in a natural sediment (80 days). Experiments were carried out with unmodified sediment, sediment amended with Na_2S (0.13 m mole) and sterilized sediment. Sterilization was accomplished in an autoclave at 121°C. $(CD_3)_3SnCl$ was synthesized from CD_3I via CD_3MgI and $SnCl_4$ (ref.10). $(CD_3)_3SnCl$ (0.2 m mole) was then incubated with a natural sediment known to be capable of methylating mercury in a sealed vessel as aboye in darkness for 80 days. 20 g of sediment made up to a volume of 30 cm^3 with water was used.

RESULTS

The results of the incubation reactions are summarized in the Table. Yields of methyl tin and methyl lead products were low, less than one per cent for the reactions with CH_3I. Definitive yields for some of these reactions are still being established. The fully saturated methyl product, $(CH_3)_4M$, is obtained for the reactions of tin and lead (0) with CH_3I. Tin (II) salts react with CH_3I to produce methyl tin species without the presence of magnesium or other reducing agent being necessary [11,12]. These results demonstrate for the first time the role of betaine, $(CH_3)_3 \overset{+}{N} CH_2C\overline{O}O.H_2O$, as a natural methylating species for a heavy metal. It is notable that CH_3CoB_{12} reacts with mercury-amino acid complexes to produce methyl mercury, apparently by a similar mechanism to its reaction with simple mercury II salts. With $Hg(meth)_2$ $(ClO_4)_2$ the reaction is approximately first order at pH 7.0.

$(CD_3)_3SnCl$ incubated in sediment over 80 days will produce a $5x10^{-2}$% yield of $(CD_3)_4Sn$; with Na_2S added to the sediments the yield is about $6x10^{-2}$%. No $CH_3Sn(CD_3)_3$ was detected in these reactions. With our GCMS system we could detect $\geq$ 5% $CH_3Sn(CD_3)_2^+$ ion (M/e 171 based on tin 120) in the presence of $(CD_3)_3Sn^+$ (M/e 174). We conclude that if $(CD_3)_3 Sn^+$ is biomethylated in our sediment system it is so to an extent of less than 5% of the disproportionation reaction.

Results for the levels of methyl mercury present in River Carron, Scotland, sediments suggest that up to a concentration of 1.8 mg g^{-1} sulphide in the sediments, CH_3Hg^+ concentration is directly proportional to sulphide concentration. Above this level of sulphide CH_3Hg^+ tends to be removed by conversion to $(CH_3)_2$ Hg by a known reaction[5] (i.e. less than 2.5×10^{-3}% overall).

TABLE METHYLATION OF TIN, LEAD AND MERCURY

	CH_3I	$(CH_3)_3N^+CH_2COO^-.H_2O$	$(CH_3)_3S^+I^-$	$(CH_3)_2S$	CH_3CoB_{12}
Sn^o	+	+	-	-	na
$SnCl_2$	+	-	+	-	+
$Sn[(COO)_2]$	+	+	+	-	na
SnS	-	-	-	-	na
Na_2SnO_3	-	-	-	-	na
Pb^o	+	-	+	-	na
$PbCl_2$	-	-	-	-	-
$PbCl_2$ Mgpresent	+	na	na	na	-
$Pb(CH_3COO)_2$	-	-	-	-	-
$Pb(NO_3)_2$	-	-	-	-	na
Pb(pen)	-[1]	na	na	na	na
Pb(eth)	-[2]	na	na	na	na
Pb(meth)	-[2]	na	na	na	na
Hg^o,hν	+[3]	na	na	na	na
Hg^o, sediment	+[4]	na	na	na	na
$HgCl_2$	-[5]	na	na	na	+
$Hg(cyst)Cl_2$,pH4.0	na	na	na	na	+[6,7]
$Hg(pen)Cl_2$,pH4.0	na	na	na	na	+[6,7]
$Hg(meth)_2(ClO4)_2$, pH1-7	na	na	na	na	+[6,8]

+ = methyl metal product detected
- = methyl metal product not detected
na = not attempted pen = penicillamine,
eth = ethionine meth = methionine cyst = cysteine

1. Product was $Pb(CH_3pen^+)I^-$
2. PbI_2 was produced
3. 50% yield
4. But yield was no greater than in the absence of CH_3I
5. A small amount of methylation occurred but due to Hg^o impurity in $HgCl_2$

TABLE continued...

6. Conc of CH_3CoB_{12} was 5.0×10^{-5} moles dm^{-3}. 100% yield based on Co. Reactions in darkness
7. Conc of Hg complex was up to 10^{-4} moles dm^{-3}
8. Conc of Hg complex was 10^{-2} mol dm^{-3}

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